EVOLUTION AND DEVELOPMENT OF TENDRILS IN BIGNONIEAE (LAMIALES, BIGNONIACEAE)[1]

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ABSTRACT

The tribe Bignonieae includes all Neotropical lianescent Bignoniaceae. The leaves of Bignonieae are generally 2- or 3-foliolate, with terminal leaflets modified into a tendril. These tendrils have varied morphologies and are thought to have been involved in the diversification of Bignonieae. Little, however, is still known about the biology and evolution of tendrils. This study investigated the evolution and development of tendril types in Bignonieae in order to further understand how changes in leaf morphogenesis led to current patterns of variation in tendril morphology. For that, we investigated the ontogeny of 11 species representing a wide diversity of tendril types (i.e., simple, trifid, and multifid) and used a recently published phylogeny of Bignonieae as the basis to reconstruct patterns of evolution for tendril types. For those analyses, we used maximum likelihood (ML) and maximum parsimony (MP) approaches, with both ACCTRAN and DELTRAN optimization schemes implemented in the latter. Ancestral character state reconstructions of tendril type suggest that the ancestral condition for the whole tribe (Core Bignonieae plus *Perianthomega* Bureau ex Baill.) is a lack of tendrils, whereas parsimony reconstructions indicate an ambiguous ancestral condition. However, all reconstructions suggest that trifid tendrils represent the ancestral condition for the Core Bignonieae. Other tendril types evolved subsequently through a series of developmental changes. Furthermore, tendril ontogenetic studies provided key information for the resolution of major ambiguities in the ancestral state reconstructions of tendril type in Bignonieae. For instance, in *Bignonia callistegioides* Cham. and *B. prieurei* DC. (simple tendrils), no traces of remnant lateral branches from a trifid-tendrilled ancestor were found, corroborating the ACCTRAN hypothesis of a simple tendril condition for the ancestor of that lineage. In *Tanaecium pyramidatum* (Rich.) L. G. Lohmann (trifid tendrils), on the other hand, we detected a pattern and rate of tendril differentiation that initially followed the same pattern seen in taxa with simple tendrils (i.e., *Cuspidaria* DC. and *Fridericia* Mart.), with a developmental delay relative to other trifid-tendrilled species, favoring a simple-tendrilled ancestor hypothesis and supporting the ML and ACCTRAN optimizations. The interpretation of the ontogenetic data in light of a robust phylogenetic framework led to specific hypotheses about the evolutionary processes and respective changes in gene regulation that may have led to the current tendril morphologies found in Bignonieae. In particular, we suggest that tendril evolution involved heterochrony and hypothesize that changes in the expression of genes that are associated with compound leaf development may have led to the diversity of tendril morphology currently observed in Bignonieae.

Key words: Ancestral character state reconstructions, Bignoniaceae, Bignonieae, compound leaves, development, evolution, heterochrony, lianas, tendrils.

Lianas account for a large percentage of the leaf biomass and floristic diversity in tropical forests (Gentry, 1991; Schnitzer, 2005), contribute ca. 44% of the woody species in some regions of the Amazon Rainforest (Pérez-Salicrup, 2001), and are important for the dynamics of forest structure and composition (Schnitzer & Bongers, 2002; Isnard & Silk, 2009). The abundance and density of lianas rise considerably with forest disturbance (Schnitzer & Bongers, 2002; Bradshaw et al., 2009), which has led to a recent increase in the interest in liana research (van der Heijden et al., 2010; Zhu & Cao, 2010; Gallagher & Leishman, 2012; Garbin et al., 2012).

The lianoid habit and associated structural modifications have intrigued biologists since Darwin (1875). Considerable research has been conducted on the stem anatomy of lianas, which include an unusual pattern of cambium activity, resulting, for

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[1] We thank Alexandre Zuntini, Marcelo Pace, and Gisele Rodrigues Costa for assistance with field collections, Harri Lorenzi for allowing us to obtain specimens from his private collection, and the Companhia Vale do Rio Doce (CVRD) for permission to collect at its natural reserve in Linhares (Espírito Santo, Brazil). We also thank Andrew Doust and Cawas Engineer for their excellent suggestions in the early stages of the development of this project, and Brad Townsley, Suzana Alcantara, Robyn Burnham, and an anonymous reviewer for their insights and comments in this manuscript. This paper is part of the Ph.D. dissertation of M.S.S.-B., and was supported by fellowships from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq grant 140470/2007-7), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES grant 4395-08-5), and a cross-disciplinary training grant from MORPH (Molecular and Organismic Research in Plant History). We also thank NSF for a Developmental Mechanisms award (# 0641696) to N.R.S., and FAPESP and CNPq (Pq-1D) for grants to L.G.L., and CAPES/PROEX for additional funding.

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doi: 10.3417/2011018

ANN. MISSOURI BOT. GARD. 99: 323–347. PUBLISHED ON 15 MAY 2014.

instance, in a xylem furrowed by phloem wedges, as observed in Bignonieae, or in the development of multiple steles, as seen in Sapindaceae (Dobbins, 1971; Dobbins & Fisher, 1986; Carlquist, 1988; Gasson & Dobbins, 1991; Gabrielli, 1993; Pace et al., 2009).

Tendrils represent another key feature associated with the evolution of the climbing habit (Gentry, 1980). Tendrils are filiform organs that have the ability to twine around other structures, representing an efficient climbing strategy (Darwin, 1875). They are defined by their function and can have different ontogenetic origins, representing the modification of leaves, leaflets, stipules, branches, inflorescences, or even roots (Putz & Holbrook, 1991). More specifically, leaf tendrils evolved several times during the evolutionary history of the angiosperms (Gentry, 1980), in particular, in families such as Colchicaceae (Liliales; Nordenstam, 1998), Polemoniaceae (Ericales; Wilken, 2004), Ranunculaceae (Ranunculales; Tamura, 1993), Papaveraceae (Ranunculales; Kadereit, 1993), Asteraceae (Asterales; Hind, 2007), Lophopyxidaceae (Malpighiales; Takhtajan, 1997), Dioncophyllaceae (Caryophyllales; Takhtajan, 1997), Fabaceae (Fabales; Darwin, 1875), and Bignoniaceae (Lamiales; Fischer et al., 2004). Interestingly, although all of these families have evolved foliar modifications to climb, such modifications are morphologically different and not homologous. However, leaf tendril types present in these distantly related plant families share an interesting characteristic: they are apparently radial structures originated from an organ with dorsoventral polarity (leaves). For instance, in the Polemoniaceae tendrils are modified leaflets (Wilken, 2004), in the Colchicaceae tendrils are leaf tips that are adapted to climb (cirrhose leaf apex; Nordenstam, 1998), and in the Dioncophyllaceae tendrils are the tips of the leaf midribs that become forked and develop a pair of hooks (Takhtajan, 1997). Despite the morphological diversity and ecological importance of leaf tendrils, very little is still known about their evolution and development outside Fabaceae, in which multiple taxa have been studied (Champagne et al., 2007; Hofer et al., 2009).

The morphology of leaf tendrils is especially diverse in Bignonieae (Bignoniaceae), which includes ca. 400 species (Lohmann & Ulloa, 2006 onwards) and represents the largest clades of Neotropical lianas (Gentry, 1991; Spangler & Olmstead, 1999; Lohmann, 2006; Olmstead et al., 2009). In Bignonieae, most tendrils arise by modifications of the terminal leaflets of the 1- to 3-ternate leaves, with the exception of *Perianthomega vellozoi* Bureau, which climbs exclusively with the aid of twinning petioles (Lohmann, 2006; Lohmann & Taylor, 2014). Tendrils across Bignonieae exhibit remarkable morphological diversity, varying from simple to bifid, trifid, or multifid, with some species possessing adhesive discs or hooks at the tip (cf. Fig. 1; Lohmann, 2006; Lohmann & Taylor, 2014). Foliar tendrils also occur in the Tourrettieae, a tribe that is restricted to the Andes and includes four species with branched foliar tendrils (Gentry, 1980; D'Arcy, 1997; Olmstead et al., 2009). Tendrils are lacking in the Paleotropical Bignoniaceae, a phenomenon thought to be associated with the lower speciation rates of the lianoid Bignoniaceae in the Old World (Gentry, 1991). In contrast, the acquisition of tendrils in the Neotropics may represent a key innovation that led to the incredible diversification of liana Bignoniaceae in that region (Gentry, 1991).

Although simple in structure, tendrils undergo complex modifications during development that result from negative phototropism, circumnutation, thigmotropism, and contact coiling (Darwin, 1875; Jaffe & Galston, 1968; Jaffe et al., 2002; Monshausen et al., 2008). During coiling, tendrils display an increase in respiration rate after contacting a support (Riehl & Jaffe, 1982). Furthermore, modifications in membrane permeability are coupled with an increase in solute efflux (Jaffe & Galston, 1968; Jaffe, 1975; Liss & Weiler, 1994; Engelberth, 2003). The appearance of cortical gelatinous fibers is also associated with tendril coiling in several plant species (Meloche et al., 2007; Bowling & Vaughn, 2009). All of these processes are coordinated by an intricate hormonal regulation (Junker, 1976; DeMason & Chawla, 2004) and are responsible for tendril development in the angiosperms.

Most developmental studies involving foliar tendrils were conducted on peas (*Pisum sativum* L.) and other species that belong to the inverted repeat-lacking clade (IRLC) of legumes (Gourlay et al., 2000; Champagne et al., 2007; Hofer et al., 2009). This clade also includes *Vicia* L., *Lathyrus* L., and *Lens* Mill., all of which bear foliar tendrils (Wojciechowski et al., 2004). Pea leaves are compound, comprised of a basal pair of stipules, one or more pairs of opposite leaflets, and a terminal branched tendril. Pea leaf primordia develop acropetally, with the tendril identity of the terminal leaflet primordium only being determined at the fourth plastochron (Gould et al., 1994).

Significant progress has been made toward a better understanding of the molecular mechanisms that regulate pea leaf development. While class 1 *KNOTTED1*-like (*KNOX1*) genes appear to control

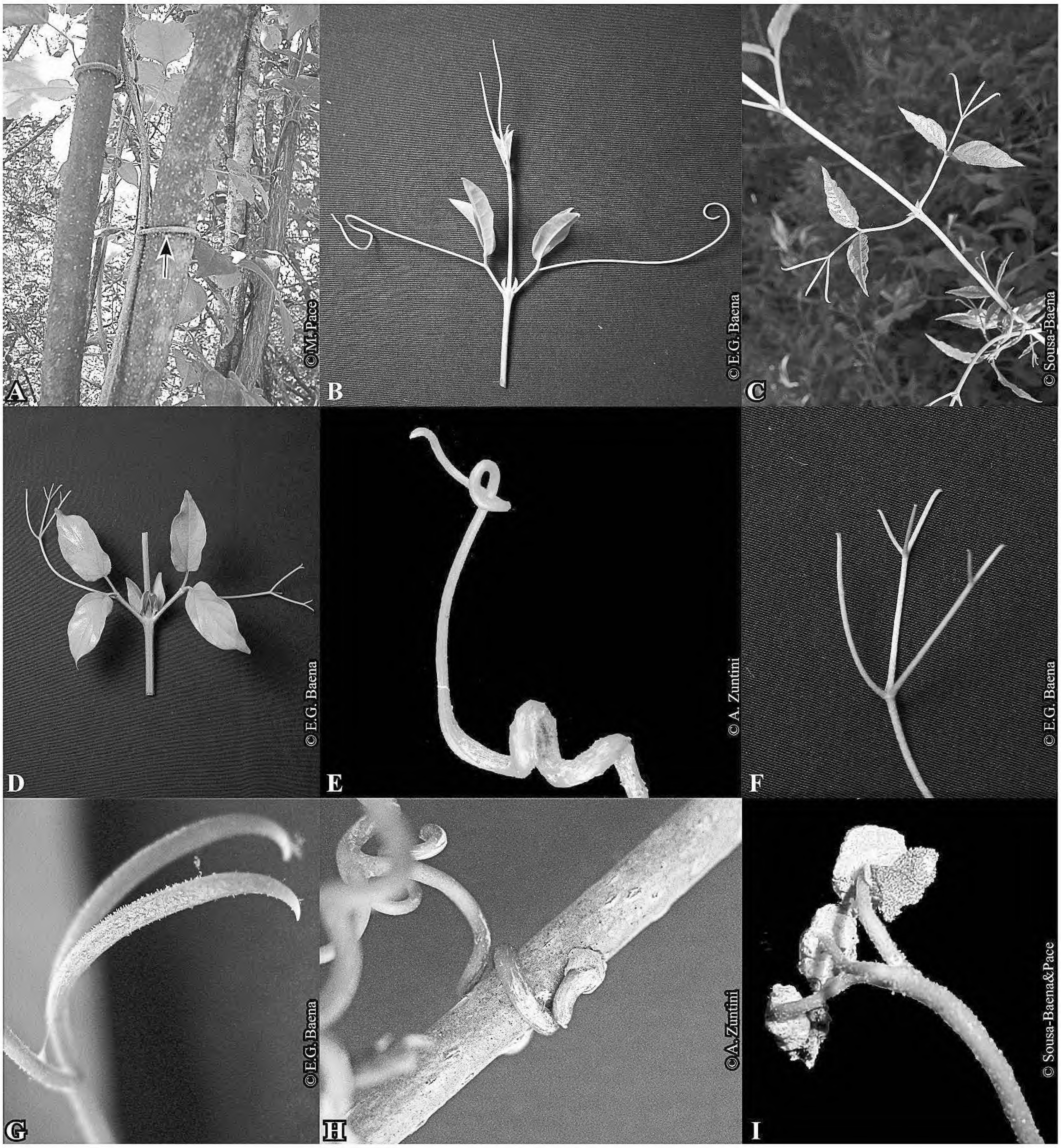

Figure 1. Sample of climbing organs found in Bignonieae. —A. *Perianthomega vellozoi* (*Pace 10*, SPF), twinning petiole (arrow). —B. *Bignonia callistegioides* (*Sousa-Baena 3*, DAV), opposite leaves composed of two leaflets and a terminal simple tendril. —C. *Dolichandra unguis-cati* (*Groppo 322*, SPF), compound leaves with a terminal trifid tendril. —D. *Amphilophium buccinatorium* (*Sousa-Baena 4*, DAV), compound leaves with a terminal multifid tendril. —E. *Adenocalymma bracteatum* (Cham.) DC. (*Lohmann 861*, SPF), detail of a simple tendril. —F. *Amphilophium buccinatorium*, detail of a multifid tendril. —G. *Dolichandra unguis-cati*, detail of a trifid tendril showing the uncinate tips. —H. *Amphilophium crucigerum* (*Pace 1*, SPF), tendril tip with adhesive disc. —I. *Tynanthus cognatus* (Cham.) Miers (*Pace 9a*, SPF), detail of a trifid tendril tip showing the adhesive discs.

the development of compound leaves across angiosperms (Sinha, 1999; Champagne & Sinha, 2004), *UNIFOLIOLATA*, a *LEAFY/FLORICAULA* ortholog, seems to regulate compound leaf development in peas (Hofer & Ellis, 1998; Champagne & Sinha, 2004). Recent studies on the garden pea (*Pisum* L.) and on the sweet pea (*Lathyrus*) indicated that the expression of *TENDRILLESS*, positively regulated by *UNIFOLIOLATA/LEAFY*, in the terminal pinnas of the wild leaf prevents leaflets from developing into a blade, leading to the formation of tendrils instead (Hofer et al., 2009).

Despite recent advances in molecular genetics of tendrils in model organisms, very little is still known

Table 1. Species sampled in the ontogenetic study, followed by their respective tendril type and phylogenetic placement. For species with polymorphic tendril types, the most common state is indicated first in the column.

Species	Tendril type	Phylogenetic placement
Bignonia prieurei DC.	simple	clade with ambiguous ancestral tendril type
B. callistegioides Cham.	simple	clade with ambiguous ancestral tendril type
Cuspidaria convoluta (Vell.) A. H. Gentry	simple	clade with a simple-tendrilled ancestor
Fridericia conjugata (Vell.) L. G. Lohmann	simple	clade with a simple-tendrilled ancestor
F. samydoides (Cham.) L. G. Lohmann	simple	clade with a simple-tendrilled ancestor
F. speciosa Mart.	simple	clade with a simple-tendrilled ancestor
Tanaecium pyramidatum (Rich.) L. G. Lohmann	bifid/trifid	clade with ambiguous ancestral tendril type
Dolichandra unguis-cati (L.) L. G. Lohmann	trifid	clade with a trifid-tendrilled ancestor
Mansoa difficilis (Cham.) Bureau & K. Schum	trifid	clade with a trifid-tendrilled ancestor
Amphilophium crucigerum (L.) L. G. Lohmann	trifid/multifid	clade with a trifid-tendrilled ancestor
Amphilophium buccinatorium (DC.) L. G. Lohmann	trifid/multifid	clade with a trifid-tendrilled ancestor

about the anatomy, morphogenesis, or evolution of tendrils in nonmodel organisms. The great diversity in tendril morphology encountered in representatives of Bignonieae, coupled with the availability of a robust phylogeny for the group (Lohmann, 2006), makes the tribe very suitable for investigating the evolution and development of tendrils.

Herein, we study the development of different tendril types in various representatives of Bignonieae and investigate the patterns of ontogenetic change within an evolutionary framework. We evaluate the historical order of change in morpho-anatomical features associated with tendrils, and suggest potential evolutionary processes that may have led to the diversity of tendrils currently encountered in the tribe. We further relate our findings to genes that are known to regulate leaf development in angiosperms, and discuss the potential role of those genes for the evolution of tendrils in Bignonieae.

Materials and Methods

ANCESTRAL CHARACTER STATE RECONSTRUCTIONS

Phylogeny

We used the single tree that resulted from the maximum likelihood (ML) analysis of a combined molecular dataset (*PepC* + *ndhF*; Lohmann, 2006) as a framework in which to map the ancestral character states of the character tendril type and estimate the historical order of appearance of the various tendril types in Bignonieae. This tree included 104 terminals of Bignonieae for which *ndhF* and *PepC* sequences were available for all taxa, plus *Perianthomega vellozoi* for which only *ndhF* sequences were available. This additional species was included in the analysis with *PepC* characters coded as missing data.

Character coding

Coding of the character "tendril type" was extracted from the morphological matrix of Lohmann (2003) and Lohmann et al. (in prep.), which included the exact same 104 terminals as the combined molecular phylogeny (Lohmann, 2006). The character tendril type was coded as "absent" when terminal leaflets modified in tendrils were lacking, as "simple" when the apices of tendrils were not parted, as "bifid" when parted twice, as "trifid" when parted three times, and as "multifid" when parted multiple times. Character coding was based on herbarium specimens that represented young and adult individuals collected in several localities. In Bignonieae, tendril type morphology is generally fixed within a species and not correlated with environmental conditions. Even though *Perianthomega vellozoi* bears simple tendrils, the tendrils of this species are twinning petioles rather than modified leaflets and so, tendrils were coded as absent in this species. The character tendril type and its respective character states were coded as discrete, non-overlapping, and multistate (for further details, see Lohmann, 2003; Lohmann et al., in prep.). When species had polymorphic tendril types (*Tanaecium pyramidatum* (Rich.) L. G. Lohmann, *Amphilophium crucigerum* (L.) L. G. Lohmann, and *A. buccinatorium* (DC.) L. G. Lohmann; Table 1), our coding used the majority rule criterion of Wiens (1999), and the most common character state was used.

Ancestral character state reconstructions

Reconstructions of the character tendril type were conducted under maximum parsimony (MP) (Maddison & Maddison, 1992) and ML assumptions (Maddison & Maddison, 2009), with tendril type treated as unordered and unweighted. Parsimony reconstructions used the all-most-parsimonious states trace option, and implemented the accelerated (ACCTRAN) and delayed (DELTRAN) trace options (Maddison & Maddison, 1992).

ONTOGENETIC STUDIES

Taxon sampling

We selected 11 species representing seven different genera (i.e., *Amphilophium* Kunth, *Bignonia* L., *Cuspidaria* DC., *Dolichandra* Cham., *Fridericia* Mart., *Mansoa* DC., and *Tanaecium* Sw.) that included a wide diversity of tendril morphologies and whose developmental data had greater potential to clarify ambiguities in tendril ancestral character state reconstructions for the ontogenetic studies (Table 1). This sampling scheme was intended to cover the breadth of variation in tendril morphology encountered in Bignonieae, as well as to help resolve ambiguities in the ancestral character state reconstructions. Specifically, we sampled six species with simple tendrils (*B. prieurei* DC., *B. callistegioides* Cham., *C. convoluta* (Vell.) A. H. Gentry, *F. conjugata* (Vell.) L. G. Lohmann, *F. samydoides* (Cham.) L. G. Lohmann, and *F. speciosa* Mart.), four species with trifid tendrils (*A. crucigerum*, *D. unguis-cati* (L.) L. G. Lohmann, *M. difficilis* (Cham.) Bureau & K. Schum., and *T. pyramidatum*), and one species with multifid tendrils (*A. buccinatorium*). Apart from representing a wide diversity of tendril types, these taxa were selected to include representatives of clades with well-established tendril conditions (i.e., homogeneous lineages in terms of tendril morphology and whose ancestral tendril condition was clear) as well as representatives of clades with ambiguous ancestral tendril types. Samples from clades with well-defined ancestors were intended to characterize the typical pattern of development for each tendril type. On the other hand, sampling of species from clades with ambiguous ancestral tendril reconstructions aimed at contributing further data toward the resolution of these ambiguities.

We used the phylogeny of Bignonieae (Lohmann, 2006), with the character tendril type mapped onto it (Fig. 2), as a basis to select taxa for the ontogenetic study. Overall, we sampled four species belonging to two genera with well-established simple tendrils (*Cuspidaria* and *Fridericia*), and three species belonging to three genera with well-established trifid tendrils (*Amphilophium*, *Dolichandra*, and *Mansoa*). In addition, we sampled representatives from two genera that presented major ambiguities in the ancestral state reconstructions of tendril type (*Bignonia* and *Tanaecium*). Our sampling scheme further included one species with multifid tendrils (*A. buccinatorium*), one species with hooked and uncinate tendril tips (*D. unguis-cati*), and two species with adhesive discs at the tendril tip (*A. buccinatorium* and *A. crucigerum*), such that a complete picture of tendril development in Bignonieae could be obtained. Vouchers for all sampled taxa are presented in Table 2.

Ontogenetic studies

Shoot apices were collected from mature plants, producing fully adult leaves. These apices were fixed under vacuum for 48 hours in glutaraldehyde and formaldehyde (Karnovsky, 1965) or 4% paraformaldehyde (Garcês & Sinha, 2009). Apices were dehydrated in a graded, ascending series of ethanol and critical-point dried with CO_2. Samples were then mounted on SEM stubs with epoxy, gold sputter-coated, and observed using SEM. For the histological investigations, shoot apices were dehydrated in a graded, ascending series of tertiary butanol and embedded in paraffin (Johansen, 1940). Embedded specimens were sectioned using a rotary microtome, then mounted on slides and stained with toluidine blue (O'Brien et al., 1964). Sections were permanently sealed with Permount (Fisher Scientific, Pittsburgh, Pennsylvania, U.S.A.).

RESULTS

ANCESTRAL CHARACTER STATE RECONSTRUCTIONS OF TENDRIL TYPE

Maximum parsimony reconstructions

Parsimony ancestral character state reconstructions of tendril type using the all-most-parsimonious states trace option support that the ancestral condition for the tribe is ambiguous, with trifid tendrils representing the ancestral condition of the Core Bignonieae clade (all Bignonieae, excluding *Perianthomega* Bureau ex Baill.). Simple tendrils have evolved at least three times from ancestors with trifid tendrils: once in the ancestor of *Adenocalymma* Mart. ex Meisn., once in the ancestor of *Bignonia*, and once in the ancestor of *Fridericia* and allies (Fig. 2). Further, at least three independent origins of multifid tendrils were reconstructed within *Bignonia*, *Manaosella* J. C. Gomes and *Neojobertia* Baill. A single evolution of bifid tendrils was hypothesized within *Tanaecium pyramidatum*, which bears both bifid and trifid tendrils. Furthermore, tendrils have been lost in 11 taxa: once in *Anemopaegma* Mart. ex Meisn. and once in *Mansoa*, both from trifid-tendrilled ancestors; twice in *Adenocalymma*, five times in *Fridericia*, once in *Xylophragma* Sprague,

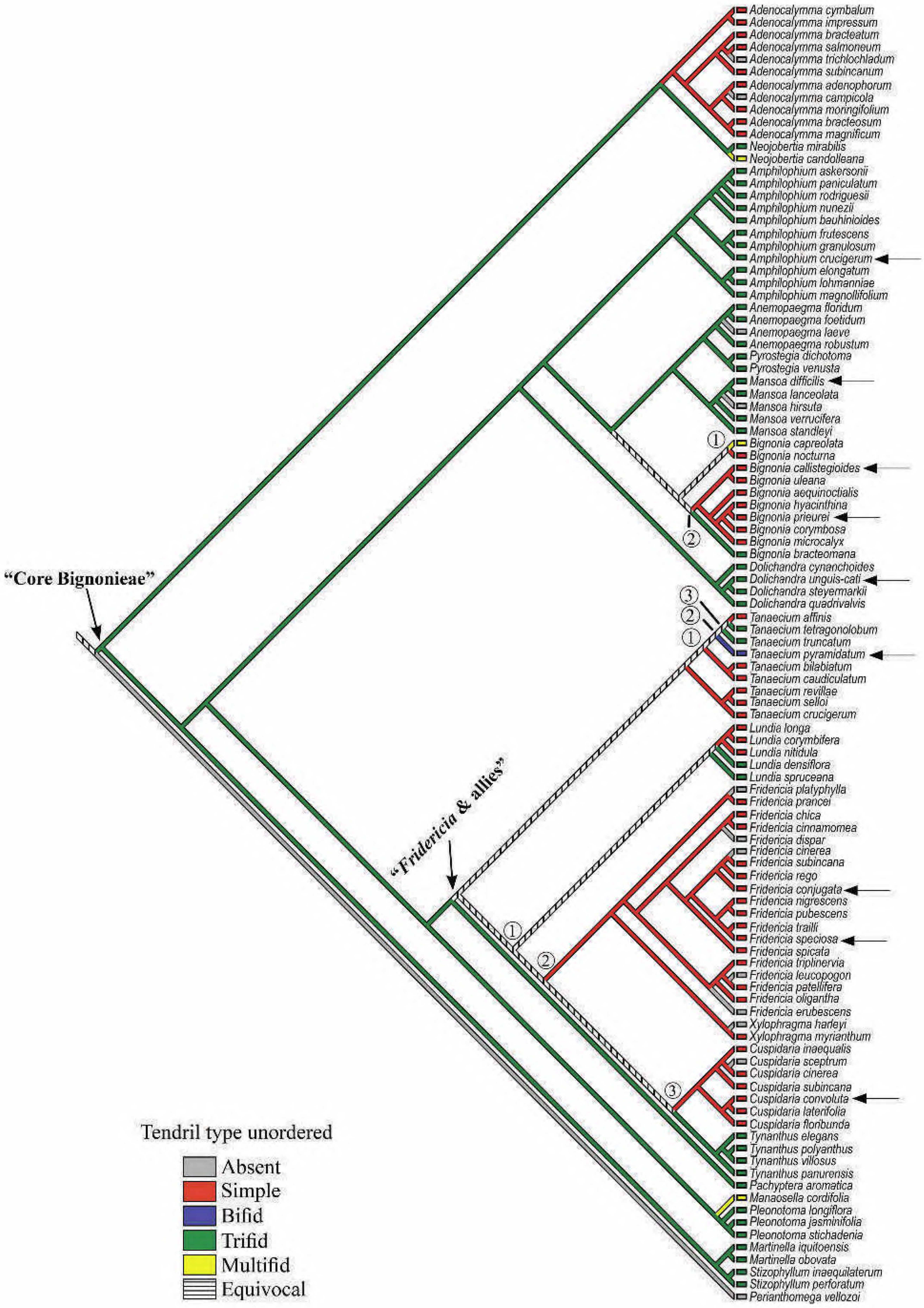

Figure 2. Single optimum maximum likelihood tree derived from the analysis of combined *ndhF* and *PepC* sequences of 104 species of Bignonieae (from Lohmann, 2006) plus *Perianthomega vellozoi*, with parsimony ancestral state reconstruction of the character "tendril type" showing all ambiguous reconstructions. Arrows indicate the phylogenetic placement of species selected for the ontogenetic study, except for *Amphilophium buccinatorium* and *Fridericia samydoides*, which were not included in this phylogeny. Clade names are modified from Lohmann (2006). Circled number = internal node number "n." Color-coded figure legend summarizes the five character states of tendril type in Bignonieae.

Table 2. Taxa sampled for ontogenetic study, with voucher detail for collectors, localities, and herbaria of deposit.

Taxon	Collection number (herbarium)	Locality
Bignonia prieurei DC.	*Zuntini 13* (CVRD, SPF)	BRAZIL. **Espírito Santo:** Linhares, Nat. Res. Companhia Vale do Rio Doce.
B. callistegioides Cham.	*Sousa-Baena 3* (DAV)	U.S.A. **California:** Davis, UC-Davis Arboretum, Terrace Garden.
Cuspidaria convoluta (Vell.) A. H. Gentry	*Pace 48* (SPF)	BRAZIL. **São Paulo:** Nova Odessa, Plantarum Bot. Gard.
Fridericia conjugata (Vell.) L. G. Lohmann	*Pace 44* (SPF)	BRAZIL. **São Paulo:** Nova Odessa, Plantarum Bot. Gard.
F. samydoides (Cham.) L. G. Lohmann	*Pace 49* (SPF)	BRAZIL. **São Paulo:** Nova Odessa, Plantarum Bot. Gard.
F. speciosa Mart.	*Pace 40* (SPF)	BRAZIL. **São Paulo:** Nova Odessa, Plantarum Bot. Gard.
Tanaecium pyramidatum (Rich.) L. G. Lohmann	*Pace 14* (SPF) *Pace 35* (SPF)	BRAZIL. **São Paulo:** São Paulo, Nat. Res. Univ. São Paulo.
Dolichandra unguis-cati (L.) L. G. Lohmann	*Groppo 322* (SPF)	BRAZIL. **São Paulo:** São Paulo, Nat. Res. Univ. São Paulo.
Mansoa difficilis (Cham.) Bureau & K. Schum.	*Pace 35* (SPF)	BRAZIL. **São Paulo:** São Paulo, Nat. Res. Univ. São Paulo.
	Zuntini 4 (CVRD, SPF)	BRAZIL. **Espírito Santo:** Linhares, Nat. Res. Companhia Vale do Rio Doce.
Amphilophium crucigerum (L.) L. G. Lohmann	*Pace 1* (SPF)	BRAZIL. **São Paulo:** São Paulo, Nat. Res. Univ. São Paulo.
A. buccinatorium (DC.) L. G. Lohmann	*Sousa-Baena 4* (DAV)	U.S.A. **California:** Davis, UC-Davis campus.

and once in *Cuspidaria*, all from simple-tendrilled ancestors.

Ambiguous reconstructions of tendril type are observed in the ancestral node of *Bignonia* and at the earliest diverging lineages of *Fridericia* and allies (which encompasses *Fridericia*, *Tanaecium*, *Lundia* DC., *Xylophragma*, *Cuspidaria*, and *Tynanthus* Miers; Fig. 2). Most of these ambiguities are resolved as simple tendrils under ACCTRAN optimization, except for the ancestor of *Lundia*, whose tendril type was reconstructed as trifid (Fig. 3A; Table 3). On the other hand, all ambiguities are resolved as trifid tendrils under DELTRAN optimizations (Fig. 3B; Table 3). The trifid tendril condition reconstructed at the ancestor of *Fridericia* and allies by DELTRAN implies six independent origins of simple tendrils within this clade (three independent origins in *Tanaecium*, one in *Lundia*, one in *Fridericia*, and one in *Cuspidaria*; Fig. 3B). On the other hand, the simple tendril condition reconstructed by ACCTRAN for the ancestor of *Fridericia* and allies implies three subsequent origins of trifid tendrils (one in *Lundia*, one in *Tanaecium*, and one in *Tynanthus*; Fig. 3A).

Overall, five evolutionary shifts to simple tendrils from ancestors with trifid tendrils are hypothesized under ACCTRAN, while nine shifts to simple tendrils from ancestors with trifid tendrils are hypothesized under DELTRAN (Fig. 3; Table 4). Furthermore, three independent evolutionary origins of multifid tendrils are hypothesized under ACCTRAN and DELTRAN, two of which are derived from ancestors with trifid tendrils and one of which is derived from an ancestor with simple tendrils under ACCTRAN optimization criteria, while under DELTRAN all multifid tendrils are derived from ancestors with trifid tendrils. In addition, at least four reversals to trifid tendrils from simple tendril ancestors are hypothesized under ACCTRAN, while a single evolution of trifid tendrils (no reversals) is hypothesized under DELTRAN, suggesting that trifid tendrils might be homologous in all lineages. A single evolutionary shift to bifid tendrils arising from an ancestor with trifid tendrils is hypothesized within *Tanaecium* under DELTRAN optimization criteria. However, ACCTRAN optimization fails to reconstruct the ancestor of an internal node of *Tanaecium* that gives rise to a heterogeneous clade that includes taxa with bifid, trifid, and simple tendrils, respectively, while DELTRAN optimization reconstructed a trifid-tendrilled ancestor for this clade.

Maximum likelihood reconstructions

ML optimizations of the character tendril type (Fig. 4) reconstructed an ancestral Bignonieae with absent

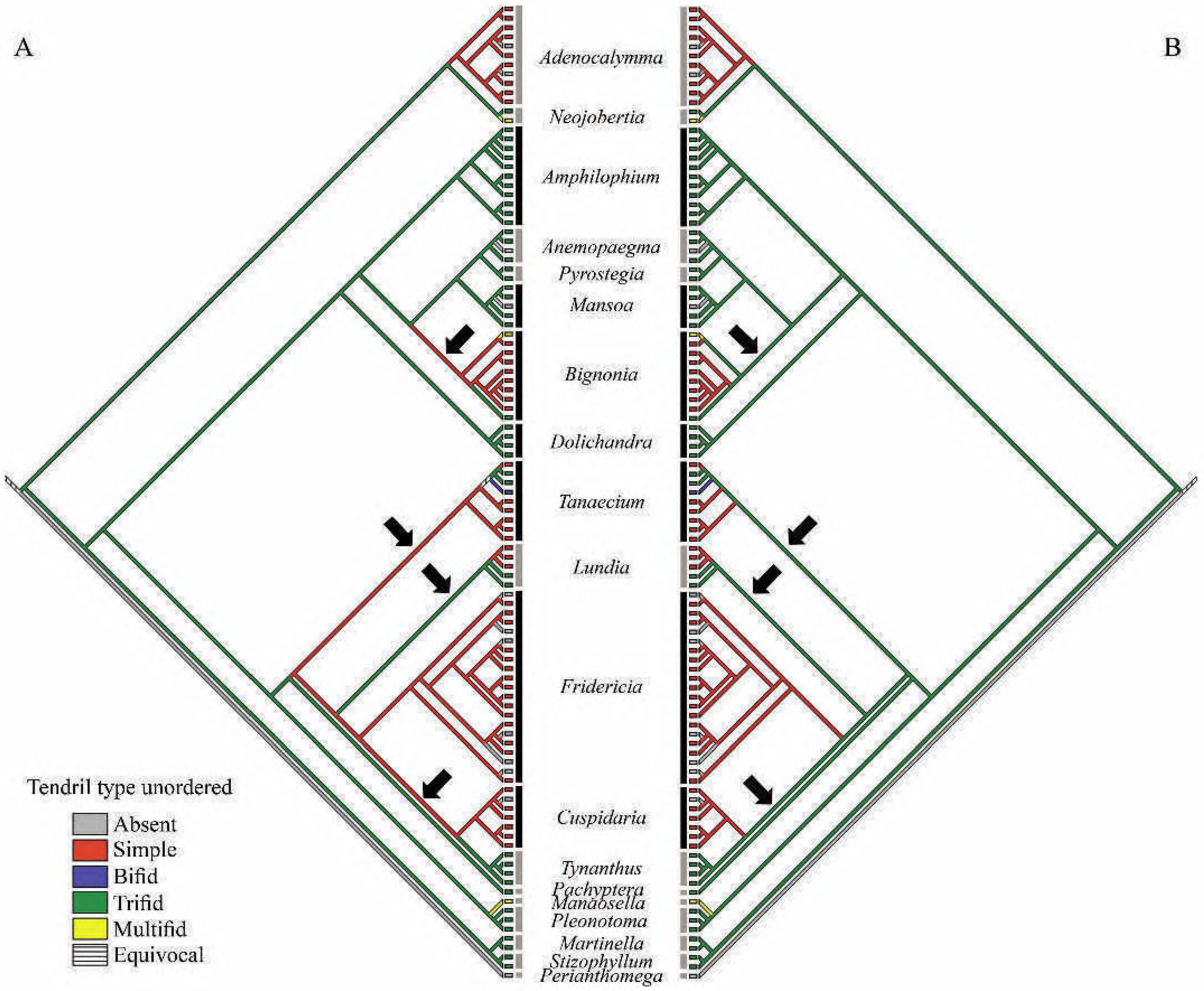

Figure 3. Single optimum maximum likelihood tree derived from the analysis of combined *ndhF* and *PepC* sequences of 104 species of Bignonieae (from Lohmann, 2006) plus *Perianthomega vellozoi*, with parsimony ancestral state reconstruction of the character "tendril type." —A. ACCTRAN optimization. —B. DELTRAN optimization. Black bars indicate the genera selected for the ontogenetic study, and black arrows indicate branches with equivocal reconstructions under the "all-most-parsimonious states" trace option. Color-coded figure legend summarizes the five character states of tendril type in Bignonieae.

tendrils (53%). The ancestral node of the Core Bignonieae was reconstructed as being trifid (78%), from which there have been seven evolutionary shifts to simple tendrils (one in *Adenocalymma*, two in *Bignonia*, one in the ancestral node of *Cuspidaria* and *Fridericia*, two in *Tanaecium*, and one in *Lundia*) from ancestors that presented trifid tendrils. One reversal to trifid tendrils was found in *Tanaecium*. Furthermore, three independent origins of multifid tendrils (in *Bignonia*, *Neojobertia*, and *Tanaecium*), and one shift to bifid tendrils (within *Tanaecium*) are also hypothesized. In addition, there have been reversals to "absent tendrils" in several taxa: once in *Anemopaegma*, once in *Mansoa*, twice in *Adenocalymma*, five times in *Fridericia*, once in *Xylophragma*, and once in *Cuspidaria*.

ML reconstructed the tendril condition for the most recent ancestor of the clade that includes *Anemopaegma*/*Mansoa*/*Pyrostegia* C. Presl/*Bignonia* as trifid (98.77%). The tendril condition at deep nodes within this clade was also reconstructed as trifid, as was the tendril condition of the ancestor of *Bignonia* (72%; see pie chart in Fig. 4). Furthermore, two independent evolutionary shifts to simple tendrils were hypothesized within *Bignonia*. On the other hand, the ML reconstruction of tendril type suggested that the ancestor of *Fridericia* and allies likely presented trifid tendrils (58%), as did the direct ancestors of *Lundia* (79.6%) and *Tynanthus* (98.2%) within this lineage. Four shifts to simple tendrils from trifid tendrils occurred, one in the ancestor of *Fridericia*/*Xylophragma*/*Cuspidaria*, one in an internal node of *Lundia*, and two in *Tanaecium* (one in the genus ancestral node and one in an internal node). A simple-tendrilled ancestor in *Tanaecium* was strongly supported (85%), with its direct ancestor being poorly supported as simple (40% simple, 25% bifid, and 32% trifid; see pie chart in Fig. 4).

Table 3. Tendril types reconstructed at the ancestral node of clades with ambiguous reconstructions under simple parsimony reconstructions. Data for the Bayesian ancestral reconstructions were retrieved from Lohmann et al. (in prep.). For the exact placement of the individual internal nodes, refer to Figure 2. AN = ancestral node; IN = internal node; "—" = inexistent node.

		DELTRAN	ACCTRAN	ML	BAYESIAN
Fridericia and allies	AN	trifid	simple	trifid	simple
	IN 1	trifid	simple	trifid	simple
	IN 2	trifid	simple	simple	simple
	IN 3	trifid	simple	simple	simple
Tanaecium	AN	trifid	simple	simple	simple
	IN 1	trifid	simple	simple	simple
	IN 2	trifid	ambiguous	simple	—
	IN 3	trifid	trifid	trifid	simple
Lundia	AN	trifid	trifid	trifid	simple
Bignonia	AN	trifid	simple	trifid	simple
	IN 1	trifid	simple	simple	simple
	IN 2	trifid	simple	trifid	simple

TENDRIL DEVELOPMENT

All species studied herein have 2-foliolate leaves, with the terminal leaflet modified into a simple, trifid, or multifid tendril. In addition, one species with hooked tendril tips (*Dolichandra unguis-cati*) and two species with adhesive discs at the tendril tip (*Amphilophium buccinatorium* and *A. crucigerum*) were also sampled. In all species studied, the leaf primordia emerge from the flanks of the shoot apex as a rounded protuberance (Figs. 5E, G, 6G), and become more triangular and flattened as development progresses (Figs. 5A, 6A, E, 7A, 10A). At the second stage of development (P2), leaflets are already defined in the leaf primordium, and the tendril is evident as the distal terminal part of the leaf primordium. Because the plants are 2-foliolate, and the tendril represents a modified terminal leaflet (Fig. 1B–D), the tendril is already discernible as the leaflets differentiate from the

Table 4. Number of evolutionary shifts for each tendril type under different ancestral character state reconstructions. Data for the Bayesian ancestral state reconstructions were retrieved from Lohmann et al. (in prep.).

	Tendril type			
	Simple	Bifid	Trifid	Multifid
ACCTRAN	5 five evolutionary shifts from an ancestor with trifid tendrils	1 one evolutionary shift from an ancestor with ambiguous reconstruction	5 one evolutionary shift at the base of Bignonieae, with four reversals from ancestors with simple tendrils	3 two evolutionary shifts from ancestors with trifid tendrils and one shift from an ancestor with simple tendrils
DELTRAN	9 nine evolutionary shifts from ancestors with trifid tendrils	1 one evolutionary shift from an ancestor with trifid tendrils	1 one evolutionary shift at the base of Bignonieae, with no reversals	3 three evolutionary shifts from ancestors with trifid tendrils
ML	7 seven evolutionary shifts from ancestors with trifid tendrils	1 one evolutionary shift from an ancestor with simple tendrils	3 one evolutionary shift at the base of Bignonieae, with two reversals from ancestors with simple tendrils	3 two evolutionary shifts from ancestors with trifid tendrils and one from an ancestor with simple tendrils
BAYESIAN	6 five evolutionary shifts from ancestors with trifid tendrils and one shift from an ancestor with multifid tendrils	1 one evolutionary shift from an ancestor with trifid tendrils	4 one evolutionary shift at the base of Bignonieae, with three reversals from ancestors with simple tendrils	3 two evolutionary shifts from ancestors with trifid tendrils and one shift from an ancestor with simple tendrils

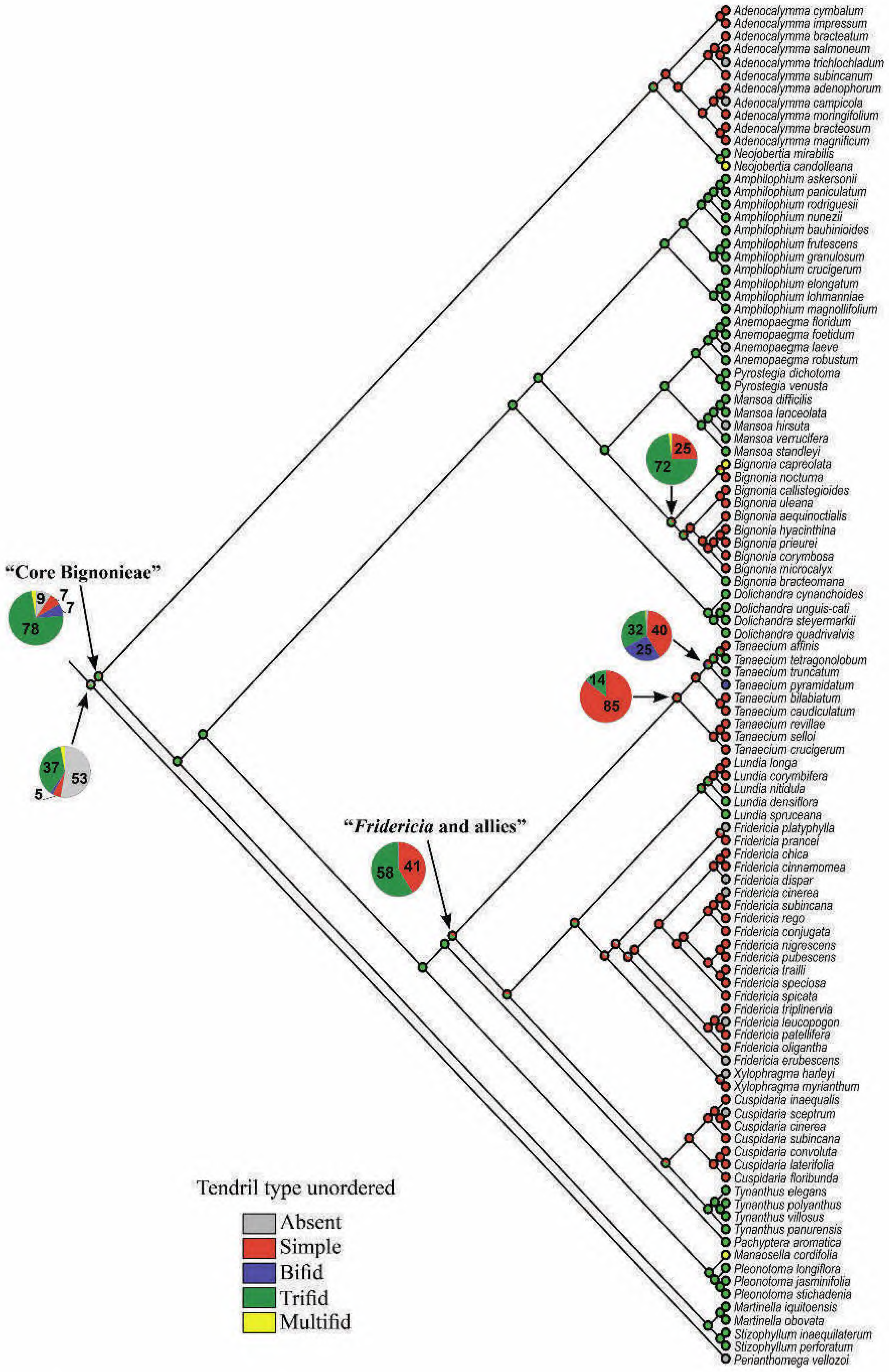

Figure 4. Single optimum maximum likelihood tree derived from the analysis of combined *ndhF* and *PepC* sequences of 104 species of Bignonieae (from Lohmann, 2006) plus *Perianthomega vellozoi*, with maximum likelihood ancestral state reconstruction of the character "tendril type." Pie charts are proportional to the likelihoods of the individual reconstructions. Numbers associated with pie charts are the likelihood values of the various tendril types. Color-coded figure legend summarizes the five character states of tendril type in Bignonieae.

base at the first stage of development (P1) (Figs. 5B, C, F, 6B, F, 7A, 8A, 10A). Independent of the tendril type, the leaf primordia always develop acropetally.

In all simple-tendrilled species studied (*Bignonia callistegioides*, *B. prieurei*, *Cuspidaria convoluta*, *Fridericia conjugata*, *F. samydoides*, and *F. speciosa*) the epidermis becomes precociously covered by

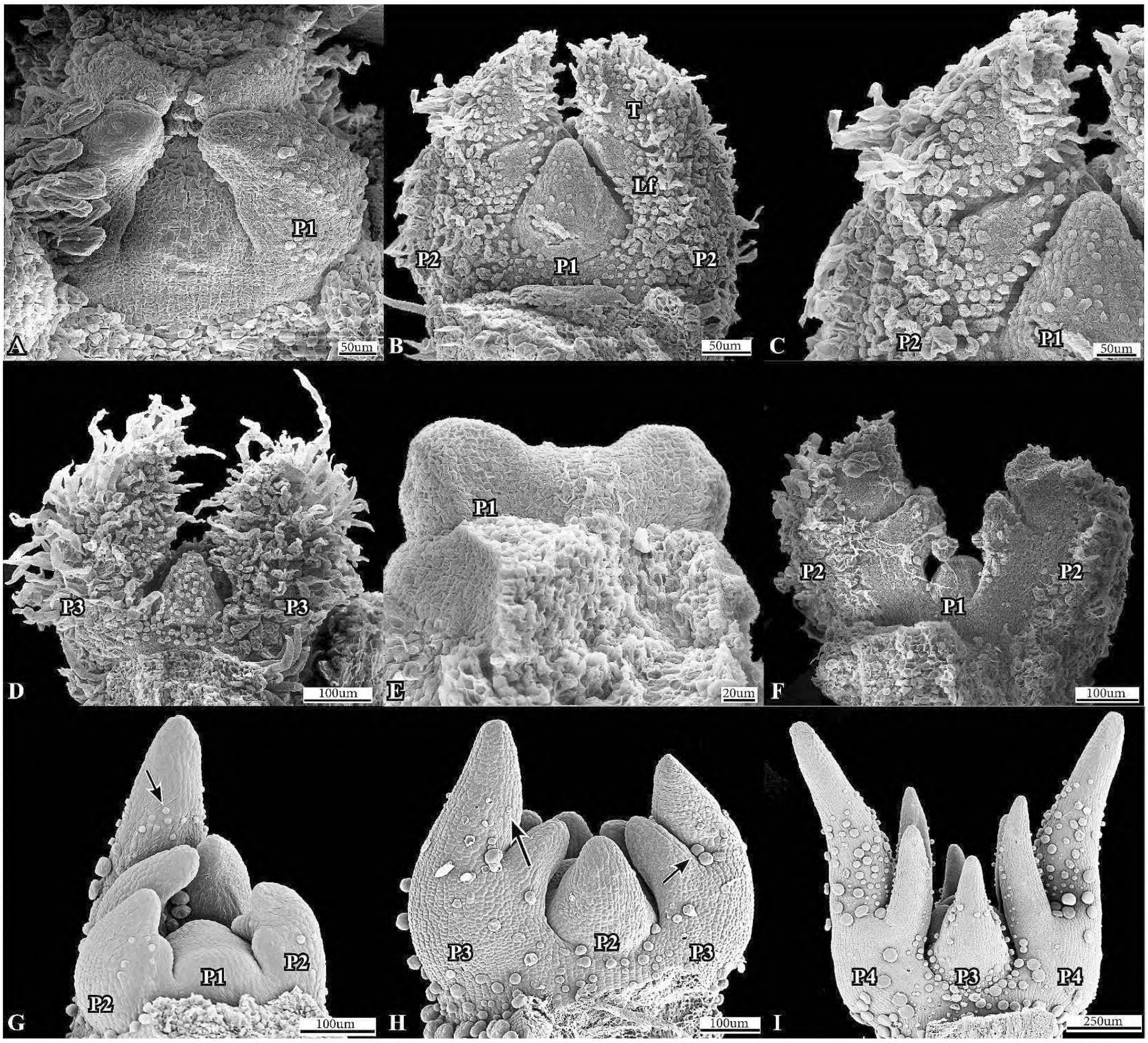

Figure 5. SEM micrographs of shoot apices illustrating the pattern of leaf development in representatives of Bignonieae with simple tendrils. A–D. *Fridericia speciosa* (*Pace 40*, SPF). —A. Shoot apex with P1. —B. Shoot apex with P2. —C. Detail of P2, showing epidermal differentiation in the adaxial surface of the tendril epidermis. —D. Shoot apex with P2 and P3, abaxial and adaxial surfaces of the P3 epidermis covered by trichomes. E, F. *Fridericia conjugata* (*Pace 44*, SPF). —E. Shoot apex with P1. —F. Shoot apex with P2. Abaxial surface of the epidermis of a P2 tendril showing a high degree of differentiation, and adaxial surface starting to develop trichomes. G–I. *Bignonia callistegioides* (*Sousa-Baena 3*, DAV). —G. Shoot apex with P1, P2, and P3. Arrow indicates trichome differentiation in the adaxial surface of P3 tendril epidermis —H. Shoot apex showing P3 in lateral view. Arrows indicate trichomes. —I. Shoot apex with P3 and P4, illustrating P4 with well-developed leaflets and tendrils and a higher degree of differentiation in the tendril epidermis. Lf = leaflet, T = tendril. Developmental stage of the leaf primordia is indicated by Pn, where "n" gives the stage of development of the primordium in question.

trichomes during leaf development (Figs. 5B, F, 6B, G, H), indicating early loss of organogenetic capacity. This differentiation wave starts at the abaxial surface and continues toward the adaxial surface (Figs. 5A, G, 6A). At P2, leaf primordia show well-developed trichomes on the abaxial surface of the epidermis as well as developing trichomes on the adaxial surface (Figs. 5B, C, 6B, H). Leaf primordia become progressively covered by trichomes, and both the abaxial and adaxial surfaces of the epidermis show a higher degree of differentiation by the third stage of development (P3) (Figs. 5D, G, 6C, D, I). By P3, most species already have tendril tips covered by trichomes, except for *B. callistegioides* in which the tendril tip is always glabrous (Fig. 5I). In *B. prieurei*, the most pubescent species sampled, the abaxial surface of the epidermis already presents a high density of trichomes at P1, while both surfaces of the epidermis are already completely covered by developing peltate trichomes at P2; these trichomes mature in P3 (Fig. 6A–D).

In all studied species with branched tendrils (*Amphilophium buccinatorium*, *A. crucigerum*, *Dolichandra unguis-cati*, *Mansoa difficilis*, and *Tanae-*

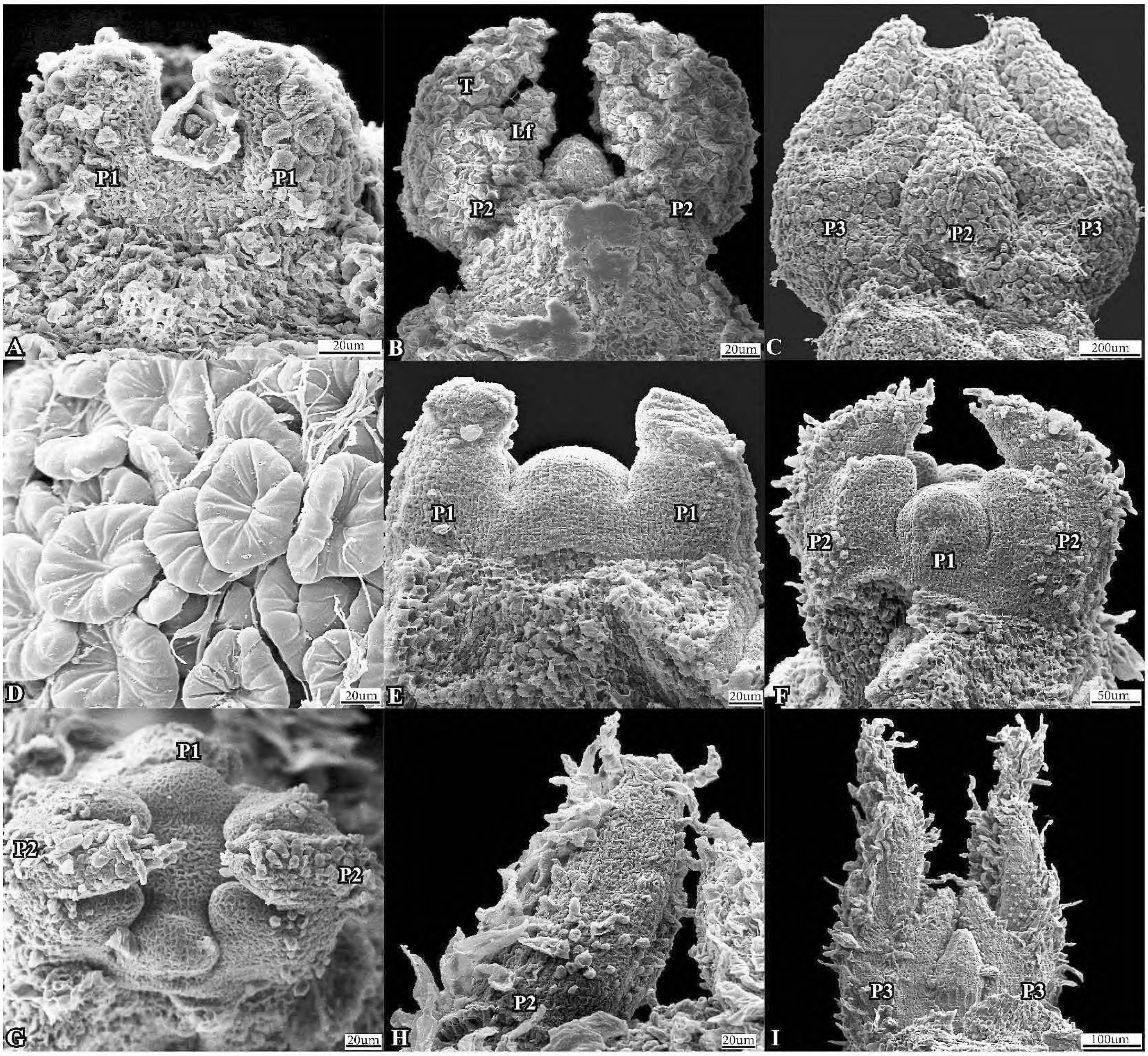

Figure 6. SEM micrographs of shoot apices illustrating the pattern of leaf development in representatives of Bignonieae with simple tendrils. A–D. *Bignonia prieurei* (*Zuntini 13*, SPF). —A. Shoot apex with P1. —B. Shoot apex with P2, showing leaf epidermis completely differentiated and covered by peltate trichomes. —C. Shoot apex with P2 and P3. —D. Shoot apex with P3, showing detail of a peltate trichome. E–I. *Cuspidaria convoluta* (*Pace 48*, SPF). —E. Shoot apex with P1. —F. Shoot apex with P2. —G. Frontal view of shoot apex, showing epidermal differentiation in P2, mainly in the abaxial surface of the tendril epidermis. —H. Shoot apex with P2 showing tendril detail and trichomes starting to develop in the adaxial surface of the epidermis. —I. Shoot apex with P2 and P3, showing both surfaces of P3 epidermis covered by trichomes. Lf = leaflet, T = tendril. Developmental stage of the leaf primordia is indicated by Pn, where "n" gives the stage of development of the primordium in question.

cium pyramidatum) leaflets are also clearly defined at P2, during which time all species start to develop trichomes on the surface of the leaf epidermis (Figs. 7A, B, 8A, 10A). As in the simple-tendrilled species, the differentiation of the epidermis is greater on the abaxial surface of the leaf primordium during the early stages of development. In *A. crucigerum*, *A. buccinatorium*, *D. unguis-cati*, and *M. difficilis*, the lateral branches of tendrils start to develop at late P2 (Fig. 7B), becoming well defined at P3 (Figs. 7A, C, 8B, 10B, C). In these species, the formation of tendril lateral branches involves tissues from the lateral flanks of the base of the tendril's primary branch (Figs. 7A–C, 8B, 10C). In taxa with branched tendrils, the rate of epidermal differentiation is generally slower than that observed in the species with simple tendrils (Figs. 7D, E, 8C–E), maintaining their organogenetic capacity for a longer period of time. Furthermore, tendril tips of the taxa with branched tendrils never develop trichomes (Figs. 7F, 8F, 10F). In *D. unguis-cati*, tendril tips curve away from the shoot apex in the fifth stage of development (P5) (Fig. 7E), becoming uncinate later, at the ninth stage of development (P9).

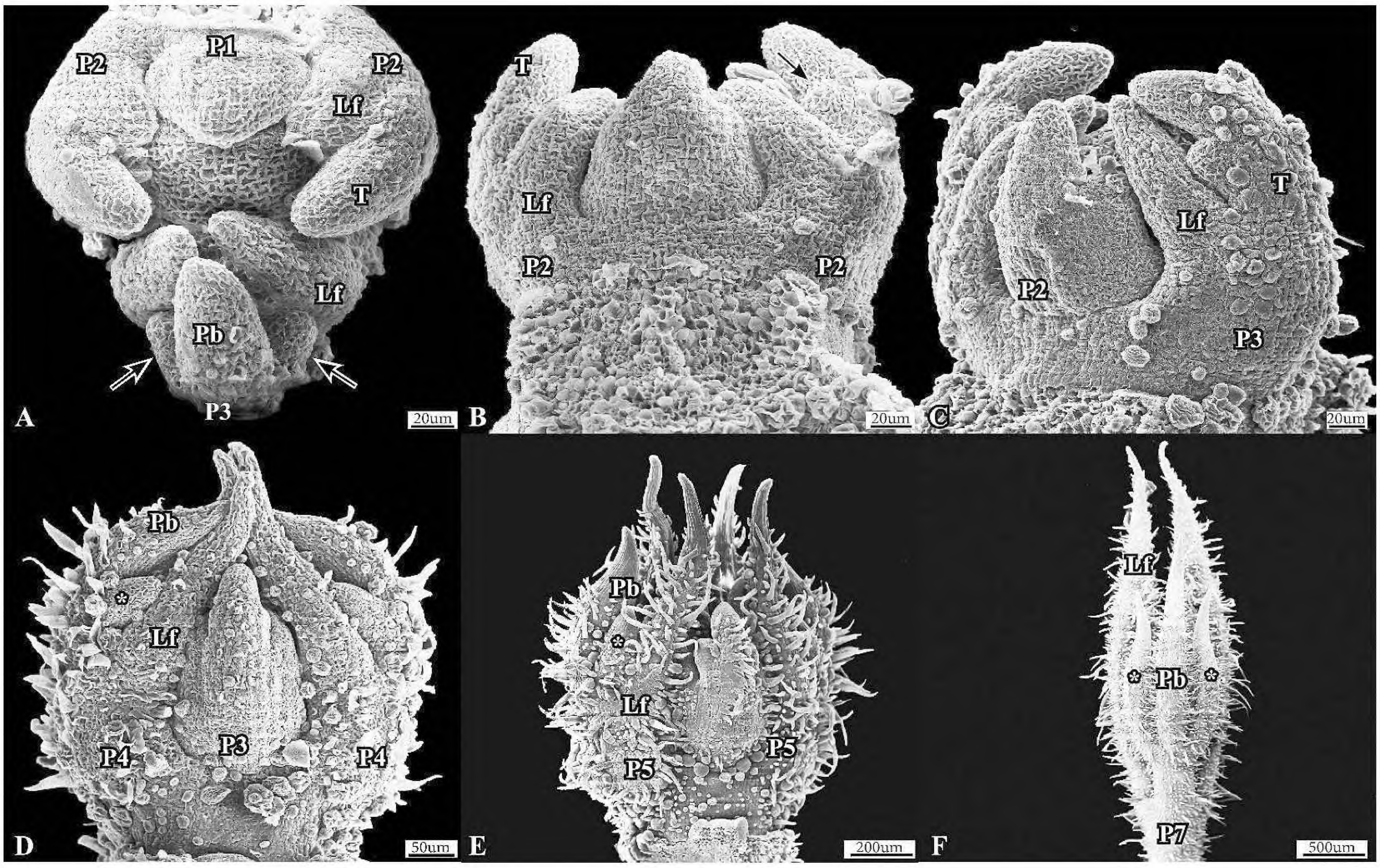

Figure 7. SEM micrographs of a shoot tip of *Dolichandra unguis-cati* (*Groppo 322*, SPF), showing the pattern of development in a trifid tendril. —A. Shoot apex with P1, P2, and P3. Arrows indicate the lateral branches of tendrils in P3. —B. Detail of early P2. Arrow indicates the tissue proliferation that will form the tendril lateral branches. —C. Shoot apex with P3, showing the epidermal differentiation in the tendrils. —D. Shoot apex with P4, showing well-developed tendril lateral branches and a higher degree of epidermal differentiation. —E. Shoot apex with P5, showing higher degree of differentiation, with primordia covered by tector and peltate trichomes. —F. Shoot apex with P7, showing tendril tips starting to become uncinate. Asterisks indicate tendril lateral branches. Lf = leaflet, T = tendril, Pb = tendril primary branch. Developmental stage of the leaf primordia is indicated by Pn, where "n" gives the stage of development of the primordium in question.

In *Tanaecium pyramidatum*, on the other hand, the tendril lateral branches emerge from the adaxial surface of the tendril primary branch. In this species, the blastozone (i.e., the organogenetic competent region located in the lateral margins of the leaf primordia; Hagemann & Gleissberg, 1996) seems contracted, occupying only the adaxial portion of the tendril primordium. Contrary to the other species that bear branched tendrils, the lateral branches of *T. pyramidatum* are not clearly defined at P3 (Fig. 8H). At this stage, the tissue protuberance that gives rise to the tendril lateral branches arises at the base of the tendril primary branch (Fig. 8H, I). An elongation of the region below the branch insertion occurs during the transition from P3 to the fourth stage of development (P4), and the lateral branches are positioned at the tip of the tendril at P4 (Fig. 8J); however, tendrils are not yet clearly defined at this stage (Fig. 8K). It is only at the sixth stage of development (P6) that the lateral branches of these tendrils become clearly visible, although lateral tendril branches remain disproportionate in size relative to the primary tendril branch at this stage (Fig. 8L).

The leaf primordia of *Tanaecium pyramidatum* show an accelerated rate of epidermal differentiation (Fig. 8G) when compared to other species with branched tendrils (e.g., *Amphilophium buccinatorium*, *A. crucigerum*, *Dolichandra unguis-cati*, and *Mansoa difficilis*). At P3, trichome density is high, reaching the tendril tip, with the region where the lateral branches arise remaining as the only glabrous portion. The degree of epidermal differentiation continues to increase during development, with the entire leaf surface (including the tip of the lateral branches of the tendril) being covered by trichomes by P6 (Fig. 8L). The other branched, tendrilled species (i.e., *A. buccinatorium*, *A. crucigerum*, *D. unguis-cati*, and *M. difficilis*) present tendril tips that remain glabrous until more advanced stages of development (Figs. 7F, 8F, 10F). The timing of differentiation of the epidermis of the tendrils of *T. pyramidatum* resembles the pattern seen in species with simple tendrils (i.e., *Bignonia callistegioides*, *B. prieurei*, *Cuspidaria convoluta*, *Fridericia conjugata*,

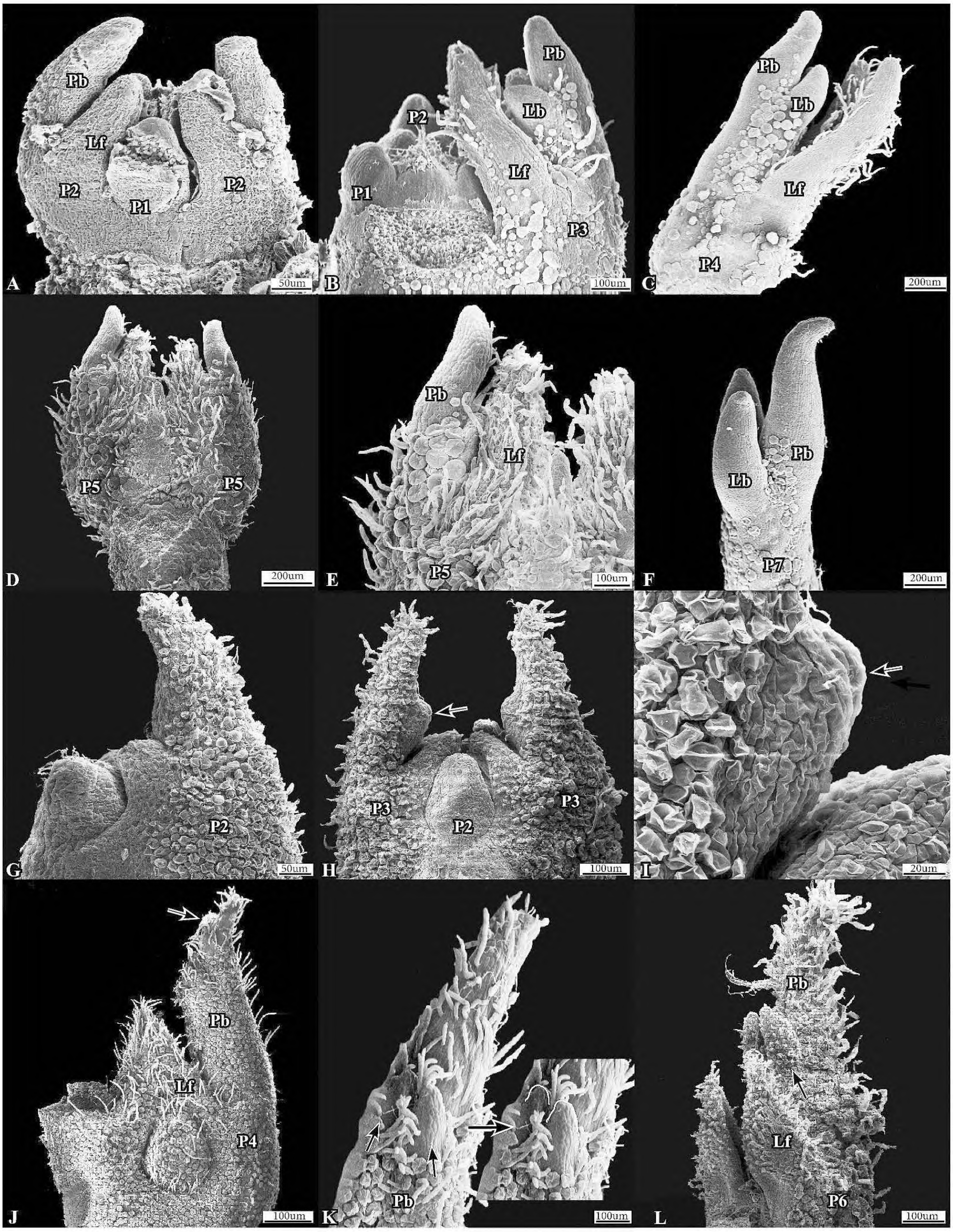

Figure 8. SEM micrographs of shoot apices illustrating the pattern of leaf development in representatives of Bignonieae with trifid tendrils. A–F. *Mansoa difficilis* (*Zuntini 4*, SPF). —A. Shoot apex with P1 and P2, showing the tendril still unbranched at P2. —B. Shoot apex with P1, P2, and P3, showing the tendril with clearly defined branches at P3. —C. Shoot apex with P4, illustrating the well-developed tendril lateral branches. —D. Shoot apex with P5, with the degree of differentiation increasing drastically from P4 (previous figure) to P5. —E. Shoot apex with P5. Detail illustrates tector and peltate trichomes on the tendril epidermis. —F. Shoot apex with P7, with tendril detail showing glabrous branched tip. G–L. *Tanaecium pyramidatum* (*Pace 35*, SPF). —G. Shoot apex with late P2. Epidermis of tendril abaxial surface showing a high degree of differentiation. —H. Shoot apex with P2 and P3. Arrow indicates the tissue proliferation in the tendril primary branch that will lead to the formation of lateral branches at P3. —I. Detail of P3, showing the beginning of lateral branch formation. Arrow indicates the tissue proliferation in the adaxial surface of the tendril. The epidermis around the tissue protuberance is covered by trichomes. —J.

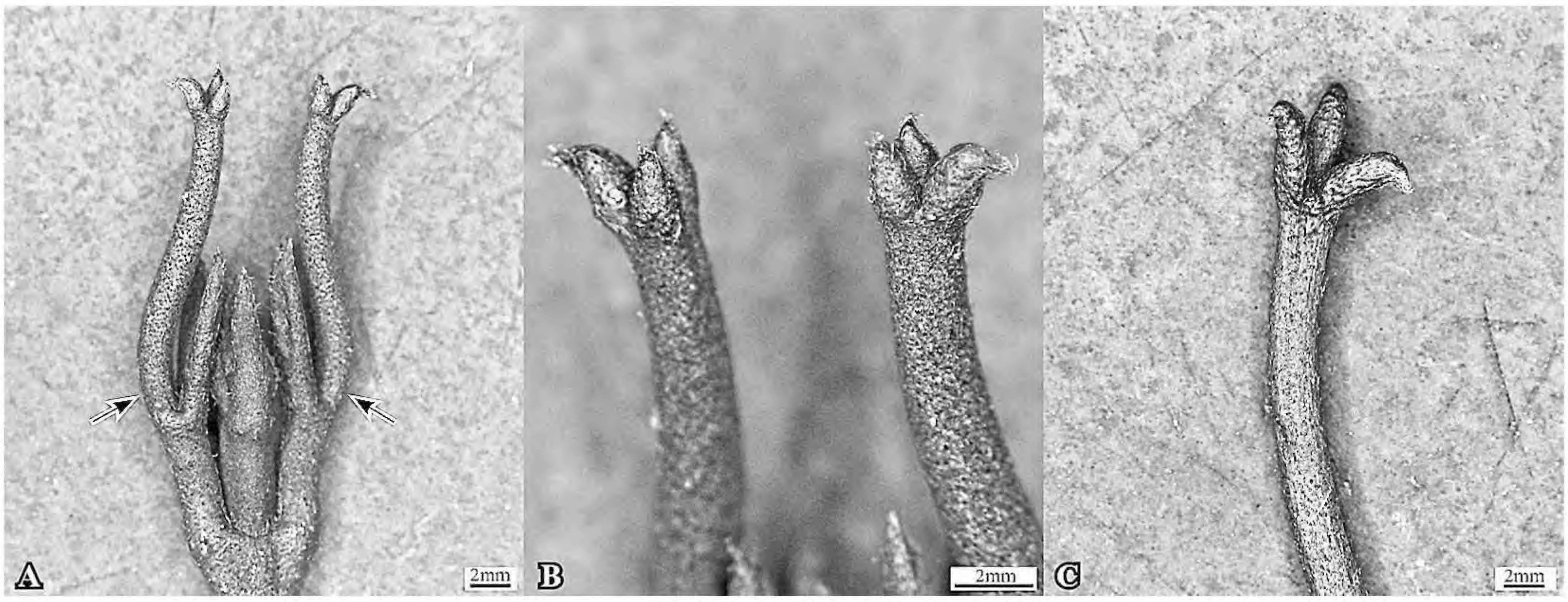

Figure 9. *Tanaecium pyramidatum* (*Pace 35*, SPF). —A. Shoot apex with P9 (arrows indicate P9). —B. Higher magnification of the tendril tip at P9. —C. P10, showing a detail of the tendril tip, and the three branches of the tendril with similar sizes.

F. samydoides, and *F. speciosa*). Although the lateral branches show a delayed onset, the three tendril branches present similar sizes by P9 (Fig. 9A, B). By the tenth stage of development (P10), the lateral branches are well developed and homogeneous in size (Fig. 9C).

Histological analyses indicate that the tissues that proliferate from tendril lateral branches have a different origin in *Tanaecium pyramidatum* when compared to the other branched, tendrilled species investigated. In *Amphilophium buccinatorium*, *A. crucigerum*, *Dolichandra unguis-cati*, and *Mansoa difficilis*, the tissue protuberance seems to originate from the epidermis and ground tissue (Fig. 11A, B). On the other hand, this tissue seems to be derived mainly from superficial layers in *Tanaecium* (Fig. 11C, D).

In *Amphilophium buccinatorium*, the only species with multifid tendrils investigated, the tertiary branches start to emerge from the primary tendril branch during P4, after the tendril lateral branches have already appeared (Fig. 10D, E). In this species, the lateral and tertiary tendril branches may develop in an asymmetrical manner, with tendril branches developing at different rates, resulting in a difference in size between the two lateral and/or tertiary branches (Fig. 10C, E). The ramifications of the secondary branches develop later, whenever present. The adhesive discs present in both species investigated for *Amphilophium* arise late in development, when leaves are already mature.

Discussion

We studied the evolution of tendrils with an integrated approach that combined ancestral character state reconstructions and detailed developmental data. The character tendril type was mapped onto a robust phylogeny of Bignonieae (Lohmann, 2006), using MP and ML assumptions. We further described tendril development in 11 species that were selected according to their phylogenetic placement and tendril morphologies (i.e., simple, trifid, and multifid tendrils). The interpretation of the ontogenetic data in light of the ancestral character state reconstructions provided key insights for the resolution of ancestral tendril types at nodes in which reconstructions were ambiguous and problematic, especially in *Bignonia* and *Tanaecium*. Below we discuss our results and raise hypotheses about evolutionary processes and molecular mechanisms that may have led to the great diversity of tendril types currently seen in Bignonieae.

ANCESTRAL CHARACTER STATE RECONSTRUCTIONS

ML reconstructed an absent tendril condition for the ancestor of the entire tribe (53%), while MP reconstructions led to an ambiguous condition for the

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←
Shoot tip with P4, showing epidermis covered by trichomes and presenting a high degree of differentiation. Tendril lateral branches not clearly defined (arrow). —K. P4, illustrating the tendril tip in detail. Arrows indicate the poorly defined lateral tendril branches. White lines delineate the limits of the lateral branches. —L. P6, showing primary branches and lateral branches (arrow) disproportionate in size and completely covered by trichomes. Lb = tendril lateral branch, Lf = leaflet, Pb = tendril primary branch. Developmental stage of the leaf primordia is indicated by Pn, where “n” gives the stage of development of the primordium in question.

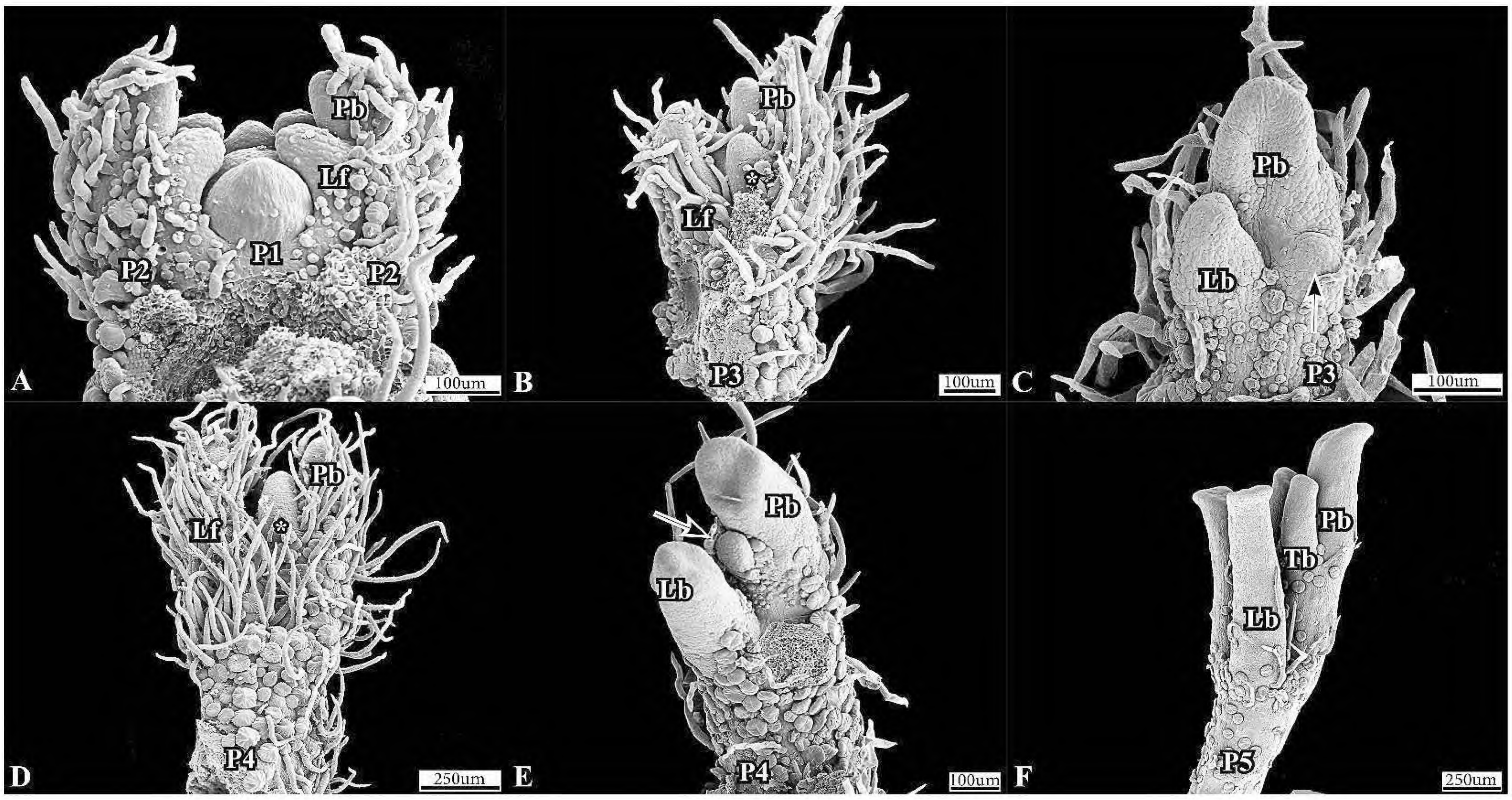

Figure 10. SEM micrographs of *Amphilophium buccinatorium* (*Sousa-Baena 4*, DAV), illustrating the development of a multifid tendril. —A. Shoot apex with P1 and P2. —B. P3 detached from shoot tip, showing the development of the lateral branches of the tendril (asterisk). —C. P3, showing the development of the asymmetrical lateral branches of the tendril (arrow). —D. P4, tendril lateral branches (asterisk) at a more advanced stage of development. Primordium covered by peltate and long tector trichomes. —E. P4, showing the asymmetrical development of the tertiary branches of the tendril (arrow). —F. P5, showing completely developed multifid tendrils. Lf = leaflet, Pb = tendril primary branch, Lb = tendril lateral branch, Tb = tendril tertiary branch. Developmental stage of the leaf primordia is indicated by Pn, where "n" gives the stage of development of the primordium in question.

ancestor of Bignonieae. Both ML and MP reconstructed a trifid condition for the ancestor of the Core Bignonieae clade (78%). The trifid condition reconstructed for the ancestor of the Core Bignonieae clade corroborates ancestral character state reconstructions using Bayesian assumptions as conducted by Lohmann et al. (in prep.). However, Lohmann et al. did not include *Perianthomega* and thus did not reconstruct the ancestral condition for the whole tribe.

ML, ACCTRAN, and DELTRAN reconstructions hypothesized a single origin of bifid tendrils (most of which derived from an ancestor that presented trifid tendrils), and three evolutionary origins of multifid tendrils. However, DELTRAN reconstructions suggest that all three origins of multifid tendrils derived from ancestors that presented trifid tendrils, while ACCTRAN, ML, and the Bayesian reconstructions from Lohmann et al. (in prep.) favor two evolutionary changes to multifid tendrils from ancestors that presented trifid tendrils, and one evolutionary shift from an ancestor that presented simple tendrils.

The number of evolutionary origins for trifid tendrils varied across methods, with a single origin being hypothesized under DELTRAN, three origins under ML, four evolutionary events under Bayesian (Lohmann et al., in prep.), and five evolutionary origins under ACCTRAN. The ancestral tendril type for the Core Bignonieae clade was reconstructed as trifid under all methods, while the ancestor of the entire tribe was ambiguous in the parsimony reconstructions, with all subsequent reversals deriving from ancestors with simple tendrils. ACCTRAN reconstruction hypothesized five evolutionary origins of simple tendrils from ancestors that presented trifid tendrils; Bayesian reconstructions hypothesized five evolutionary origins of simple tendrils from ancestors that presented trifid tendrils (Lohmann et al., in prep.), while ML reconstructions hypothesized seven origins. Conversely, DELTRAN favored a much higher number of evolutionary origins for simple tendrils (nine), all of which were derived from ancestors that presented simple tendrils.

Overall, ACCTRAN and ML ancestral state reconstructions show similar patterns of tendril evolution. In contrast, DELTRAN optimizations consistently led to divergent scenarios. Most of the ambiguity associated with the ACCTRAN and DELTRAN reconstructions is associated with the ancestral tendril condition reconstructed for *Bignonia*, and with the tendril types reconstructed for the earliest diverging lineages of *Fridericia* and allies. In all instances, the same ancestral tendril types were reconstructed by ACCTRAN and Bayesian approach-

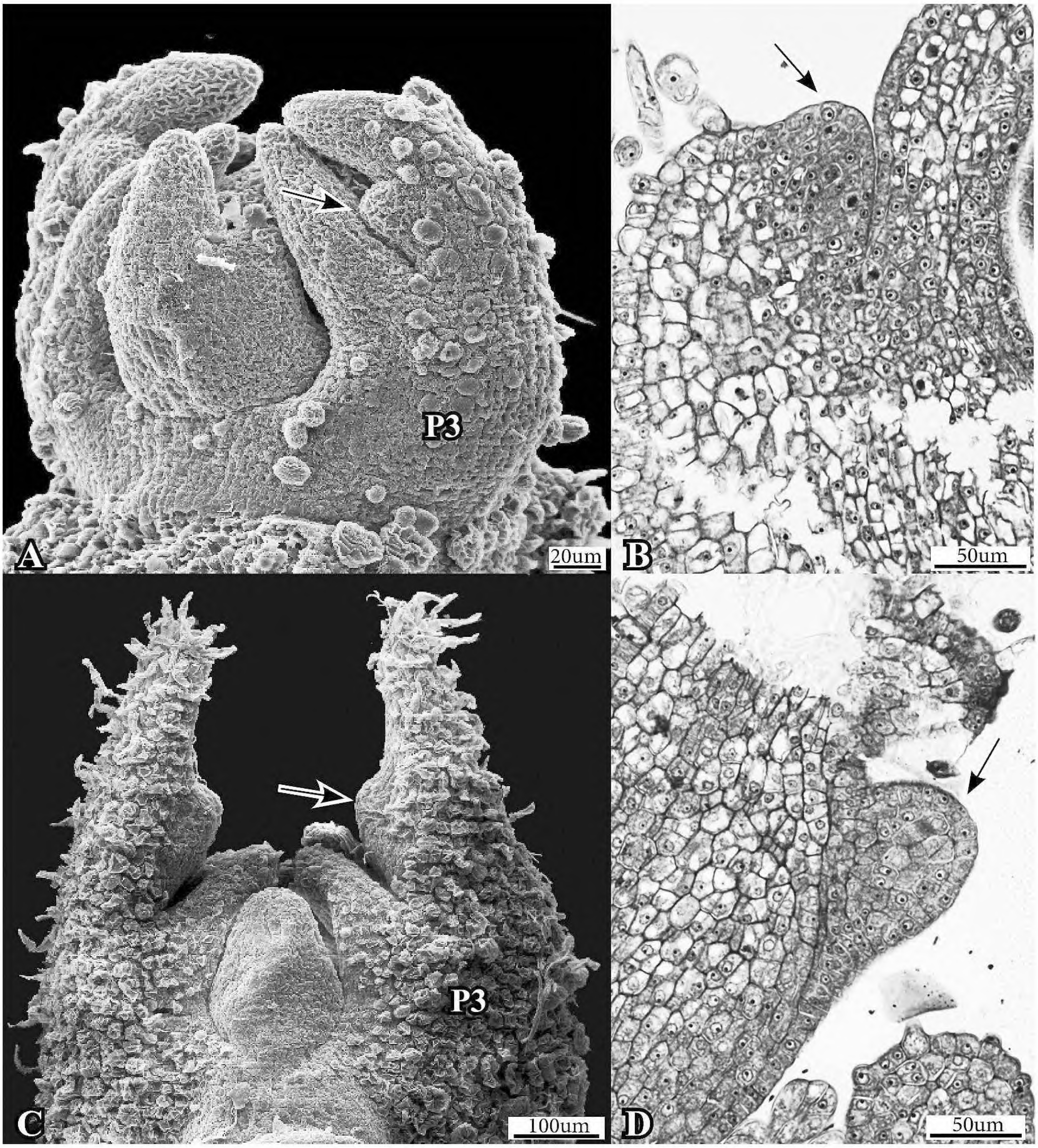

Figure 11. SEM micrographs and histological sections of leaf primordium of *Dolichandra unguis-cati* and *Tanaecium pyramidatum*. A, B. *Dolichandra unguis-cati* (*Groppo 322*, SPF). C, D. *Tanaecium pyramidatum* (*Pace 35*, SPF). —A, C. Shoot apices with P3, showing trifid tendril with tissue proliferation that will lead to the formation of the tendril lateral branches (arrows). —B, D. Longitudinal sections of the tissue bulge that will form the lateral tendril branches. —B. Formation of the tendril lateral branches (arrow) involving the division of cells in the epidermal and ground tissue. —D. Tissue that will give rise to tendril lateral branches (arrow) seems to have originated mainly from superficial layers. Developmental stage of the leaf primordia is indicated by Pn, where "n" gives the stage of development of the primordium in question.

es. On the other hand, ML hypothesized a trifid ancestor for *Bignonia* and *Fridericia* and allies, which is in agreement with the DELTRAN reconstructions (Table 3). While ACCTRAN optimizations accelerate changes toward the root of the tree, increasing the number of reversals, DELTRAN optimizations delay changes away from the root, increasing independent gains (Swofford & Maddison, 1987). Although DELTRAN and ACCTRAN optimizations were created as tools of equal value to infer evolutionary patterns, there has been a preference for resolving ambiguous reconstructions using ACCTRAN (Agnarsson & Miller, 2008). This preference is associated with the notion that reversals are more likely than parallelisms and that the loss of complex traits is more likely than parallel gains (Pinna, 1991; Agnarsson & Miller, 2008). In this study in particular, ACCTRAN and ML reconstructions favored multiple evolutionary origins of trifid and simple tendrils, while DELTRAN favored a single evolutionary event for trifid tendrils and a much higher number of evolutionary steps for simple tendrils (at least nine). Even though the latter scenario is in agreement with the concept that loss of complex traits is more likely than repeated gains, in general all other reconstructions (ACCTRAN, ML, and Bayesian) and ontogenetic data do not favor this hypothesis.

The differences encountered between the various methods of ancestral character state reconstruction were not surprising, given that each of these methods is based on a different set of criteria and assumptions. For example, MP prioritizes the smallest number of evolutionary changes and favors the least complex explanations for an observation. On the other hand, model-based approaches, such as ML, favor the most probable explanations for a given dataset, which may or may not represent the most parsimonious explanations (Cunningham et al., 1998; Omland, 1999; Pagel, 1999). In general, different evolutionary scenarios are reconstructed with the employment of distinct reconstruction methods and assumptions, changing the way we interpret how evolutionary events have occurred and what mechanisms are responsible for them (Losos, 1999). Integrated approaches that combine ancestral character state reconstructions with ontogenetic data allow us to refine our evolutionary hypotheses as well as to pose subsequent hypotheses about the evolutionary processes and genetic mechanisms that can lead to the patterns of morphological diversity seen in extant species.

TENDRIL DEVELOPMENT

Blastozone activity

All species with simple tendrils studied here showed very similar patterns of development, including an early loss of organogenetic capacity and precocious epidermal differentiation during leaf development. On the other hand, leaf primordia of all species with branched tendrils studied (*Amphilophium buccinatorium*, *A. crucigerum*, *Dolichandra unguis-cati*, and *Mansoa difficilis*) present slower rates of epidermal differentiation and longer organogenetic capacity than species with simple tendrils. Furthermore, these species bear tendril branches that start to appear at late P2 (Fig. 7B), becoming clearly defined at P3 (Figs. 7A, C, 8B, 10B, C). Species with branched tendrils also show glabrous tendril tips at late stages of development.

In *Tanaecium pyramidatum*, on the other hand, the three tendril branches become clearly visible only at P6, and a delay in the development of the tendril lateral branches is observed when compared to the other species with branched tendrils. In addition, *T. pyramidatum* shows an accelerated rate of epidermal differentiation when compared with the other species with branched tendrils.

In most species with trifid tendrils (*Amphilophium crucigerum*, *Dolichandra unguis-cati*, and *Mansoa difficilis*) the pattern of activity of the blastozone during the formation of lateral branches of tendrils seems to be a reiteration of the activity pattern used to form leaflets. The blastozone is located in the same relative position (marginal) in the tendril primary branch as compared to the leaf primordium and shows a similar pattern of fractionation. Thus, maintenance of the organogenetic competent tissue (blastozone) with similar properties in the primary tendril branch may be responsible for the morphogenesis of the most common leaf type in Bignonieae. Likewise, the ramification pattern of the multifid tendrils of *A. buccinatorium* is not random. These tendrils are trifurcated several times, representing a reiteration of the initial pattern of activity found in the blastozone during leaflet formation.

Conversely, the blastozone is restricted to a portion of the adaxial surface of the tendril primary branch in *Tanaecium pyramidatum*. The epidermal differentiation program is activated earlier during the development of tendrils in *T. pyramidatum* than it is in the other trifid-tendrilled species of Bignonieae. On the other hand, the program to promote cell cycle activity in the blastozone is delayed in *T. pyramidatum* relative to other species with trifid tendrils. Thus, it is possible that the delay seen in the development of the lateral branches of *T. pyramidatum* may result from a disassociation between the differentiation/indeterminacy program and the genetic program that promotes cell cycle activity in the blastozone. Hence, when the cell-division cycle activity is triggered in the blastozone at P3, the intense epidermal differentiation that has already progressed restricts the blastozone to a small portion of the adaxial surface of the tendril. Consequently, tendril lateral branches in this species are derived from a smaller pool of meristematic cells, resulting in the formation of lateral and primary tendril branches that are disproportional in size (Fig. 8K, L).

Genes involved in the regulation of the blastozone activity

The degree of blade elaboration in compound leaves is determined by the complex and delicate balancing of tissue differentiation and indeterminacy that is dynamically maintained during leaf development. Several genes regulate these processes, and the most general mechanism to maintain indeterminacy seems to involve the expression of *KNOX1* genes (Smith et al., 1992; Bharathan et al., 2002). More recently, *TCP* genes (another family of transcription factors) were found to also play an important role in the control of indeterminate identity during leaf development (Efroni et al., 2008). In particular, *TCP* genes were shown to play a major role in promoting tissue differentiation in the simple leaves of *Arabidopsis thaliana* (L.) Heynh. (Efroni et al., 2008). Furthermore, increases in *TCP* expression during the development of complex leaves of tomato result in a premature differentiation of leaf margins, leading to simplification of the leaf blade (Ori et al., 2007). One possible hypothesis to explain the interaction between *KNOX1* and *TCP* genes during leaf development is that the acquisition of *KNOX1* expression during compound leaf development, coupled with a fine adjustment of the expression of *TCP* genes, in a developmental stage-dependent manner, would regulate the degree of leaf differentiation as a whole and specify the regions in the blastozone that form lateral outgrowths (Floyd & Bowman, 2010; Koenig & Sinha, 2010).

Assuming that *KNOX1* and *TCP* genes perform essentially the same functions in Bignonieae, some changes in the expression of *TCP* genes must have occurred in *Tanaecium pyramidatum*. More specifically, the expression of *TCP* genes in earlier stages of development or an increased *TCP* expression may have caused early primordium differentiation and restriction of organogenetic competent tissue, resulting in a delay in the onset of the tendril lateral branches, as well as a delay in the rate of development.

In *Pisum sativum*, leaf development is controlled by *UNIFOLIOLATA/LEAFY* instead of *KNOX1* genes. In other species placed in the same clade (IRLC), leaf development is also controlled by *LEAFY*, with tendril development being directly associated with *TENDRILLESS* expression, a class I *HDZIP* gene (Hofer et al., 2001, 2009). Bignonieae tendrils are modified terminal leaflets that are similar to pea tendrils, suggesting that *LEAFY* could also represent a major regulator of leaf development in representatives of the tribe. However, *STM* (*KNOX1* gene) is expressed during leaf development in representatives of Bignonieae (Sousa-Baena et al., 2014), indicating that it is unlikely that *LEAFY* is the primary regulator of leaf development in representatives of Bignonieae.

ARP genes are responsible for the establishment of adaxial identity in developing leaves (Waites et al., 1998). In simple-leaved plants, *ARP* genes are expressed in the developing leaves, repressing the expression of *KNOX1* (Champagne & Sinha, 2004; McHale & Koning, 2004). However, in compound leaves of tomato, *KNOX1* and *ARP* have overlapping expression patterns, suggesting that both genes regulate meristematic functions during leaf development in this species (Kim et al., 2003). Silencing of *ARP* genes results in the formation of needle-like leaflets in several species of plants (Waites & Hudson, 1995; Schneeberger et al., 1998; Timmermans et al., 1999; Byrne et al., 2000; McHale & Koning, 2004). Even though needle-like leaflets are morphologically similar to tendrils, studies on the function of *ARP* genes in pea tendril development showed that this gene does not play an important role in the formation of tendrils in this species (Tattersall et al., 2005). Since pea leaf development is controlled by *LFY/FLO* rather than *KNOX1* genes, it is reasonable to assume that genes that interact directly with *KNOX1* also do not play key roles during leaf development in peas. However, *KNOX1* genes are expressed during leaf development of representatives of Bignonieae (Sousa-Baena et al., 2014), suggesting that interactions between *STM* and *ARP* genes may be involved in tendril determination in representatives of this clade. Moreover, other genes that regulate the establishment of abaxial/adaxial identity (e.g., *HD-zipIII* genes, *YABBY*, and *KANADI*) may play important roles in tendril development as well.

Alternatively, changes in the hormonal regulation during key leaf developmental stages might be responsible for the delayed development of tendril branches observed in *Tanaecium pyramidatum*. In particular, the production of auxin maxima induces the formation of leaflet primordia along the leaf margin (Barkoulas et al., 2008; Koenig et al., 2009; Floyd & Bowman, 2010; Koenig & Sinha, 2010). These auxin peaks are associated with the promotion of cell cycle activity and a shift in the plane of cell division, events that together lead to outgrowth of lateral organs (Koenig & Sinha, 2010). Hence, it is possible that a delayed auxin maximum might be associated with the delayed development of tendril lateral branches in *T. pyramidatum*.

INTEGRATING TENDRIL DEVELOPMENTAL DATA AND ANCESTRAL CHARACTER STATE RECONSTRUCTIONS

Four of the six simple-tendrilled species studied (*Cuspidaria convoluta*, *Fridericia conjugata*, *F. samydoides*, and *F. speciosa*) are placed within lineages with well-established simple tendrils. The remaining two species (*Bignonia callistegioides* and *B. prieurei*) are within lineages whose ancestral tendril types are ambiguous. In addition, four species with branched tendrils are found in clades whose ancestors are unambiguously reconstructed as trifid (*Amphilophium buccinatorium*, *A. crucigerum*, *Dolichandra unguis-cati*, and *Mansoa difficilis*; Figs. 2–4). On the other hand, *Tanaecium pyramidatum*, another species with branched tendrils, is placed within a clade whose ancestor is ambiguously reconstructed as simple or trifid (Figs. 2–4; Table 1).

Most representatives of Bignonieae present 2-foliolate leaves, with the terminal leaflet modified into a tendril (Lohmann & Taylor, 2014). Ancestral state reconstructions of tendril type indicated that leaves without tendrils represent the ancestral condition in the tribe, which was followed by multiple evolutionary origins of different tendril types. However, ancestral state reconstructions of tendril type, based on different algorithms, led to ambiguous reconstructions in several nodes (Table 3). In particular, the ancestral tendril condition for *Bignonia* is ambiguous, as well as the ancestral tendril condition for the *Fridericia* clade (internal node in Fig. 2) and those of several lineages included herein. Because *Tanaecium* represents the earliest diverging lineage within the *Fridericia* clade, the resolution of the ancestral tendril condition for this genus is critical for a better understanding of the patterns of tendril evolution in all subsequent lineages.

As far as *Bignonia* is concerned, the tendril condition of the most recent ancestor of this clade was reconstructed as trifid under ML and DELTRAN optimizations, but as simple under ACCTRAN. Bayesian reconstructions corroborate the ACCTRAN reconstructions (Lohmann et al., in prep.). Ontogenetic studies of two species of *Bignonia* with simple tendrils (*B. callistegioides* and *B. prieurei*) indicated a developmental pattern for these species that is very similar to that of species of *Cuspidaria* and *Fridericia*, both of which belong to lineages with well-established, simple tendrils (i.e., homogeneous lineages in terms of tendril morphology, including simple-tendrilled species exclusively, and a clear simple-tendrilled direct ancestor). In particular, these species of *Bignonia* do not present any traces of lateral tendril branches that could be reminiscent of a trifid-tendrilled ancestor during development. Thus, the developmental data can be interpreted as support for early evolution of simple tendrils in the history of this genus, suggesting that the ancestral condition for *Bignonia* is simple tendril, corroborating the ACCTRAN and Bayesian ancestral character state reconstructions (Lohmann et al., in prep.).

As far as *Tanaecium* is concerned, DELTRAN optimizations reconstructed a trifid tendril condition for the ancestor of this clade, while ML and ACCTRAN reconstructed a simple tendril condition for the ancestor of this clade. Bayesian reconstructions (Lohmann et al., in prep.) corroborate the ML and ACCTRAN reconstructions. In the latter, the appearance of trifid tendrils in *Tanaecium* would represent subsequent reversal from simple tendrils. In this case, the simple-tendrilled ancestor would have suppressed the genetic program that leads to the formation of lateral branches (i.e., cell cycle activity in the blastozone). Ontogenetic studies of *T. pyramidatum* (a species with trifid tendrils) indicated that this genetic program was indeed reactivated in this species. However, this activation was delayed when compared to other trifid-tendrilled species. Furthermore, the pattern and rate of tendril differentiation in *T. pyramidatum* followed those found in lineages with well-established, simple tendrils (i.e., *Cuspidaria* and *Fridericia*), which includes early loss of organogenetic capacity. In other words, earlier stages of development in this species followed those of simple-tendrilled taxa, while later stages followed those of trifid-tendrilled species, leading to a mosaic of traits that are typical of simple-tendrilled taxa (i.e., rapid differentiation of the epidermis of the leaf primordia), and trifid-tendrilled taxa (i.e., the retention of organogenetic capacity in the primary branch of the tendril). While the reacquisition of trifid tendrils in this genus is homoplastic, it probably reflects some kind of underlying homology to form lateral branches that must have been retained from ancestral patterns of blastozone fractionation (Hall, 2003; Rutishauser & Moline, 2005). On the other hand, the retention of characteristics that are typical of simple tendrils in the earlier stages of development supports the hypothesis that its direct ancestor would have presented simple tendrils, thus favoring the ML, ACCTRAN, and Bayesian ancestral state reconstructions.

EVOLUTIONARY PROCESSES THAT MAY HAVE LED TO THE CURRENT DIVERSITY IN TENDRIL MORPHOLOGY

The emergence of a new area of scientific research, evolutionary developmental biology (Gilbert, 2003; Love & Raff, 2003), has prompted the reassessment of several classical concepts, such as homology, homoplasy, heterochrony, homeosis, heterotopy, and novelty (Muller & Wagner, 1991; Li & Johnston, 2000; Baum & Donoghue, 2002; Cronk, 2002; Hall, 2003; Rutishauser & Moline, 2005; Wagner et al., 2005). In particular, new findings about shared developmental pathways encountered among organisms that are not necessarily closely related led to new definitions of several of these classical concepts (Hall, 2003; Laubichler & Maienschein, 2003; Cracraft, 2005; Brigandt & Griffiths, 2007; Jaramillo & Kramer, 2007; Wagner, 2007). The excitement generated by the possibility of linking gene function to phenotype has brought new interpretation to classical concepts. This facilitates the analysis of developmental data in light of the newly available phylogenies and allows a better understanding of the evolution of development (Raff, 2000). Under this approach it is possible to infer how the dissociation between developmental modules could lead to evolutionary shifts, through processes like heterochrony, heterotopy, and homeosis (Li & Johnston, 2000; Baum & Donoghue, 2002; Cronk, 2002; Wagner et al., 2005).

As stated above (see discussion under *Blastozone activity*), the pattern of activity of the blastozone during the formation of tendril lateral branches in trifid-tendrilled species in Bignonieae seems to represent a reiteration of the pattern seen during the formation of leaflets. In the trifid-tendrilled species, the blastozone is situated in the same relative position (marginal) in the tendril primary branch when compared with the leaf primordium. On the other hand, the activity and position of the blastozone are different in *Tanaecium pyramidatum* when compared to those of other trifid-tendrilled species, with the lateral branches showing a delayed onset and growth rate.

In this context, it seems likely that the evolution of trifid tendrils in *Tanaecium* may have resulted from paedomorphosis by post-displacement (i.e., a delay in the onset of development of some organ or structure in the organism; Alberch et al., 1979). It is important to note that the term post-displacement was coined by zoologists (Alberch et al., 1979), whose study of organisms presents determinate growth and hence, if the heterochronic structure kept the same developmental rate seen in the ancestor, it would be smaller in the adult animal (Alberch et al., 1979; McNamara, 1986). However, plants have indeterminate growth, and sexual maturity cannot be used as an offset reference (Li & Johnston, 2000). Adult tendrils of *Tanaecium* have branches of equal size (Fig. 9C), which might result from a delayed developmental onset being consumed by the inherent capacity of plants for indeterminate growth.

Changes in the timing, rate, or spatial patterns of gene expression have been shown to represent one of the underlying causes of phenotypic variation (Raff, 1996; Tautz, 2002). Thus, the use of the term heterochrony was extended and is now also used to describe changes of the developmental timing at the molecular and genetic levels, apart from changes at the morphological level (Carroll, 2000). Heterochronic genes that specifically control developmental timing have been isolated in both plants and animals (Ambros & Horvitz, 1987; Slack & Ruvkun, 1998; Hunter et al., 2003; Chuck et al., 2007; Efroni et al., 2008; Sharbel et al., 2010).

In *Tanaecium*, heterochronic processes, such as a delay of the auxin maxima formation in the blastozone, coupled with an earlier onset of *TCP* expression, could be responsible for the evolution of trifid tendrils. Under this hypothesis, opposing types of heterochrony would be acting on tendrils, with post-displacement of auxin maxima formation and earlier onset of differentiation, resulting in a post-displaced development of tendril lateral branches. It is likely that much of the phenotypic variation that we see in extant species may have arisen through heterochronic processes (Gould, 1992). Indeed, the evolution of one single structure in a certain group of plants can be the result of several, and sometimes contrary, heterochronic processes acting in concert (Alberch et al., 1979; Li & Johnston, 2000).

Our results suggest that simple tendrils have evolved from trifid tendrils several times in Bignonieae. In these plants the epidermal differentiation is rapid, and the organogenetic competence appears to be lost early in development. Thus, changes in spatio-temporal patterns or levels of *TCP* expression could also be related to the precocious differentiation of the epidermis in simple-tendrilled species, and ultimately responsible for the arising of simple tendrils in Bignonieae. Indeed, it seems that a gradual loss of organogenetic competent tissue in leaf primordia over evolutionary time may have led to the current diversity of tendril morphology.

Conclusion

The interpretation of tendril ontogenetic data in light of a robust phylogenetic context revealed some interesting evolutionary trends. First, *Dolichandra* and *Mansoa* present trifid tendrils and arise within lineages whose immediate ancestors already had trifid tendrils. In these genera, the three tendril branches were already defined at P3. Second, *Tanaecium* belongs to a lineage whose immediate ancestors had simple tendrils. In this genus the three tendril branches are only clearly defined at P6 and a considerable delay in tendril development is observed when compared to species with trifid tendrils whose immediate ancestors had trifid tendrils. In this species, tendril development is heterochronic, with the evolution of trifid tendrils involving the maintenance of simple tendril characteristics that were present in its ancestor, as well as the reacquisition of traits that are typical of trifid tendrils. Third, *Bignonia* presents the same developmental pattern as *Cuspidaria* and *Fridericia*, two genera that belong to lineages with well-established, simple tendrils, indicating that the evolution of simple tendrils happened early in the evolutionary history of these lineages.

Developmental studies contributed data that greatly improved the resolution of the ancestral character state reconstructions of tendril type in Bignonieae. These data corroborated ACCTRAN and Bayesian reconstruction in all instances and generally corroborated ML reconstructions, but did not support DELTRAN reconstructions in any instance. To date, detailed comparisons across methods of ancestral character state reconstructions are still limited, indicating the importance of using multiple methods when reconstructing ancestral character states. Furthermore, integrative approaches, i.e., combining data from multiple sources, are more likely to lead to more accurate ancestral character state reconstructions and more robust evolutionary hypotheses. Ideally, those hypotheses should be tested at a molecular level, involving spatio-temporal changes in gene expression that may have led to the patterns of phenotypic variation currently observed. Gene expression studies using in situ RNA hybridizations and comparing species with varied tendril morphologies are bringing new insights on the role of *STM*, *ARP*, and *LEAFY/FLORICAULA* genes in the regulation of tendril development in Bignonieae (Sousa-Baena et al., 2014). Further studies using gene silencing and gain-of-function approaches could also contribute important information for a better understanding of the function of those genes in Bignonieae.

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